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MEASUREMENTS OF THE VOLATILE PRODUCTION OF APPLES[1]

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INTRODUCTION

The investigations of Brooks and Cooley (*1, 2*)[2] and Brooks, Cooley, and Fisher (*3, 4, 5*) on apple scald control with oiled wrappers and shredded oil paper indicate that organic vapors arising from apples are directly responsible for the development of this physiological disorder. However, there is no satisfactory evidence at present to show which one or ones of the many volatile constituents emanating from apples are responsible for storage scald.

If apple volatiles are truly responsible for the development of apple scald, quantitative measurements of the organic vapors arising from apples in the fall might indicate the relative amount of scald that one could expect at any given stage of maturity and set of storage conditions.

Since it is generally considered that the organic gaseous products arising from apples are directly responsible for apple scald, it seemed desirable to determine their magnitude. Measurements of volatile emanations and respiration were accordingly made on McIntosh apples (*Malus pumila* Mill.) picked at regular intervals through two harvesting seasons. The influence of temperature, storage atmosphere, and vapors from ripe apples on the rate of respiration and volatile production were also studied. Special precautions were taken to make sure that vapors from previously harvested fruit did not come in contact with the experimental apples.

REVIEW OF LITERATURE

The most complete analysis of the odorous constituents arising from apples was made by Power and Chesnut (*25*). By suitable chemical procedures they found amyl ester of formic, acetic, caproic and caprylic acids, acetaldehyde, and ethyl alcohol. Later, they (*26*) discovered geraniol, an aliphatic terpene alcohol, in the peel of McIntosh apples. It is of interest that they were unable to detect the presence of amyl valerate which is generally known as "apple oil."

Probably the most widely known of the organic metabolic gases arising from fruits is ethylene. Elmer (*6*) noticed that apple vapors

[1] Received for publication November 15, 1943.

[2] Italic numbers in parentheses refer to Literature Cited, p. 293.

inhibited the sprouting of potatoes. Later, Smith and Gane (*27*) found that apple emanations greatly decreased the growth of pea seedlings. By using large numbers of absorbents they noted that bromine, ozone, fuming nitric acid, and fuming sulfuric acid removed the active agent from apple vapors. All of these chemicals react readily with unsaturated hydrocarbons. Eventually, Gane (*7*), Hansen and Christensen (*16*), and Niederl, Brenner, and Kelley (*24*) identified ethylene as a constituent of vapors emanating from apples. Hansen and Christensen (*16*) found no acetylene, propylene, or butylene. Niederl, Brenner, and Kelley (*24*) were also unable to find propylene or butylene. Consequently, the influence of apple vapors as observed by Elmer (*6*) and the epinastic effects noted by Gane (*10*) are generally considered to be due to ethylene.

Quantitative measurements of total volatiles arising from apples have been made by Gane (*11*) and Kidd and West (*21*) by employing a complete combustion technique. Large differences were found in the volatile respiration ratios among different varieties of apples. Gane (*11*) shows that these differences in ratios are due mainly to variations in the production of organic vapors. Kidd and West (*21*) observed that as the temperature was lowered from 15° to 3° C. the rate of respiration and volatile production dropped markedly. Their data with Bramley's Seedling apple indicate that although the curve in the production of volatiles follows respiration rather closely, it lags behind respiration and reaches its peak a number of days after the respiratory climacteric is reached.

Recently, Gerhardt and Ezell (*14*) measured the volatiles from Golden Delicious apples by absorbing the emanations in concentrated sulfuric acid and subsequently oxidizing them with ceric sulfate. At 65° F. the climacteric in respiration preceded the peak in volatile production by 6 days. After this peak in organic vapors had been reached, the rate dropped rapidly. It was later realized by Gerhardt (*13*) that ethylene was not absorbed appreciably by concentrated sulfuric acid.

Acetaldehyde is one of the most prevalent of organic emanations from fruit and has been measured frequently from apple and pear tissue. Harley and Fisher (*18*), Harley (*17*), and Gerhardt and Ezell (*15*) have stressed the importance of acetaldehyde as the causal agent of scald and core break-down of pears. Gerhardt (*13*), working with apple tissue, found that acetaldehyde accumulated in areas affected by soft scald. Miller (*22*) however, points out that—

> * * * high acetaldehyde content of apple peel may be due in part to its production by cells ruptured in the paring process, but there is also a tendency for the acetaldehyde to accumulate in peel affected either by mechanical injury to the cells or by abnormal physiological conditions of the fruit.

The quantities of ethylene arising from apples is small, although there is undoubtedly much variation among varieties. Gane (*8*) estimates that approximately 1cc. of ethylene is produced by an apple in its entire life. Nelson (*23*) has shown that at 20° C. McIntosh apples liberate their largest quantities of ethylene about 5 days after the respiratory peak. Their maximum output was about 2.0 mg. of ethylene per kilogram of fruit per day at this temperature.

The influence of ethylene on the rate of respiration of preclimacteric fruit is well known. Gane (*12*) and Kidd and West (*19, 20*), working

with bananas and apples, respectively, noted that the climacteric in respiration could be brought about by low concentrations of ethylene. Gane (*12*) found that bananas which were in their postclimacteric phase were uninfluenced by ethylene so far as the respiratory rate was concerned.

Kidd and West (*19*) and Smock (*29*) have been able to induce the climacteric in sunripe apples by passing vapors from ripe fruits over the immature ones. The vapors from ripe apples, however, were unable to influence the rate of respiration of apples which were already respiring at an accelerated rate.

METHODS

The procedure employed for the measurement of apple volatiles was one devised by Gerhardt and Ezell (*14*) with a few modifications. The absorbent used was 35 ml. of concentrated sulfuric acid (specific gravity, 1.84) placed in a suitable absorption tower. The towers were made from 2-foot sections of 24-mm. pyrex glass tubing, stoppered into 500-ml. suction flasks. The towers were then about half filled with pyrex glass beads (2 to 6 mm). The air containing the apple vapors passed through the towers during the 20- or 24-hour determinations at the rate of approximately 28 liters per hour. After the absorption period was completed, the tower was removed from the suction flasks and the beads were allowed to fall into the flasks. The sides of the tower were washed down with distilled water, care being taken to collect all washings in the suction flask. Then a known volume of standard ceric sulfate was added and the solution was made up to 250 to 300 ml. with distilled water. The flasks were then placed in an autoclave and allowed to remain there from 6 to 8 hours at 15 pounds pressure (approximately 121° C.). At the end of that time the excess ceric sulfate was titrated with approximately 0.1 N ferrous sulfate, orthophenanthroline ferrous sulfate being used as the indicator (*28*). The exact normality of ferrous sulfate was determined daily by standardizing it against the previously standardized ceric sulfate (*28*) which is exceedingly stable (*31*).

Gerhardt and Ezell (*14*) reported that their method was satisfactory after duplicating their results very closely when known quantities of acetaldehyde were oxidized. Since they knew that acetaldehyde was an organic volatile arising from fruits, they believed that the apple emanations could be determined by the same procedure. It was thought, however, since esters are also produced by apples, that it might be wise to standardize their procedure against an ester. Ethyl acetate was selected as the ester to be used.

A diagram of the apparatus in which ethyl acetate was placed is shown in figure 1. With the mercury in the positions indicated in the diagram, the apparatus was weighed to the nearest tenth of a milligram on an analytical balance. Then a sample of ethyl acetate vapor was drawn off by passing air through this container in the direction indicated in figure 1, and absorbed in the tower containing the sulfuric acid. As the air moved through the container for the ester, the mercury was forced up into the side nipples. If any of the mercury splashed past the nipples it was either caught in the trap or in the bulb containing the volatile liquid. Except for necessary

rubber connections all tubing was either of glass or copper. After an hour or two the container holding the ethyl acetate was removed from the system, and by tipping this volatile holder properly the mercury was allowed to flow back into the positions shown in figure 1. The container was immediately reweighed and the loss in weight represented the ethyl acetate absorbed by the sulfuric acid. Known volume of ceric sulfate were added as previously described and after digestion and titration, the milligrams of ceric sulfate reduced per milligram of ester oxidized were calculated.

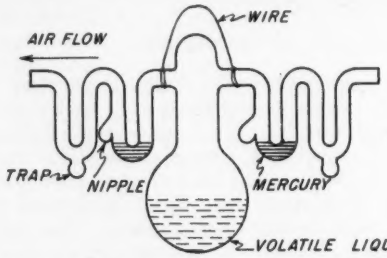

FIGURE 1.—Diagram of container used to measure the weight of ethyl acetate lost to an absorption tower.

The influence of the length of the digestion period and temperatures during digestion are given in tables 1 and 2. From the data shown in table 1 it can be seen that no readily reproduceable reduction-oxidation values are obtained by boiling for periods up to 21 hours. Gerhardt and Ezell (*14*) found that acetaldehyde could be completely oxidized in 2 hours at 98° C. However, by digesting this solution in an autoclave at 15 pounds' pressure at intervals of 2 hours and titrating the excess ceric sulfate after each period, it was possible to determine the time required to completely oxidize this ester at this temperature. By comparing the results presented in table 1 with those in table 2 it can be seen that after 6 hours at 15 pounds' pressure the reduction-oxidation values were much greater than those resulting after 15 hours of boiling.

The variations in the reduction-oxidation ratios are not considered significant in view of the crudeness of the apparatus used. It was not the author's desire to determine the absolute reduction-oxidation ratio for ethyl acetate, but rather to find how long a period and how high a temperature would be necessary to oxidize the apple emanations which are absorbed in sulfuric acid.

TABLE 1.—*Influence of time and temperature on the oxidation of ethyl acetate with ceric sulfate*

Ethyl acetate (milligrams)	Oxidation		Ceric sulfate (0.1060 N) used	Ceric sulfate reduced	Reduction-oxidation ratio
	Time	Temperature			
	Hours	*°C.*	*Milliliters*	*Milligrams*	
86.7	15	88	50	1,353.5	15.6
90.3	15	88	50	1,284.9	14.2
130.1	15	88	50	1,599.8	12.3
93.6	15	98	50	1,509.4	16.1
117.8	15	98	50	1,765.1	15.0
114.5	21	98	75	2,058.2	18.0

TABLE 2.—*Influence of time and temperature on the oxidation of ethyl acetate with ceric sulfate under 15 pounds pressure*

Ethyl acetate (milligrams)	Oxidation		Ceric sulfate, 0.1060 N., used	Ceric sulfate reduced	Reduction-oxidation ratio
	Time	Temperature			
	Hours	*°C.*	*Milliliters*	*Milligrams*	
56.9	2	121	50	1,523.1	26.77
	2	121	25	73.4	1.29
	2	121	25	8.6	.15
Total 56.9	6	121	100	1,605.1	28.21
59.1	2	121	50	1,527.1	25.84
	2	121	25	144.2	2.44
	2	121	25	12.0	.20
Total 59.1	6	121	100	1,683.3	28.48
53.9	2	121	50	1,467.3	27.22
	2	121	25	50.8	.94
	2	121	25	5.7	.11
Total 53.9	6	121	100	1,523.8	28.27
64.8	2	121	50	1,687.9	26.05
	2	121	25	122.6	1.89
	2	121	25	10.6	.16
Total 64.8	6	121	100	1,821.1	28.10

After observing the results shown in tables 1 and 2 it seemed quite probable that complete oxidation of apple volatiles by ceric sulfate might not take place after 2 hours of boiling. The results shown in table 3 were obtained after oxidizing the organic vapors arising from one-half bushel of ripe McIntosh apples at room temperature.

TABLE 2.—*Influence of time and temperature on the oxidation of ethyl acetate vapors with ceric sulfate*

Oxidation		Ceric sulfate, 0.1261 N, used	Ceric sulfate reduced per day	Ceric sulfate reduced per kilogram of fruit per day
Time (hours)	Temperature			
	°C.	*Milliliters*	*Milligrams*	*Milligrams*
2	121	50	996.0	135.8
2	121	25	136.1	18.6
2	121	25	13.3	1.8
2	121	25	6.6	.9
2	121	25	0	0
Total 10		150	1,152.0	157.1
2	98	25	368.5	50.2
2	121	25	667.3	90.9
2	121	25	56.4	7.7
2	121	25	10.0	1.4
2	121	25	6.6	.9
Total 10		125	1,108.8	151.1
2	98	25	368.5	49.0
2	121	25	640.8	85.1
2	121	25	49.8	6.6
2	121	25	10.0	1.3
2	121	25	10.0	1.3
Total 10		125	1,079.1	143.3

From the results obtained it seems quite clear that boiling for 2 hours is hardly sufficient for complete oxidation of apple volatiles. The data also indicate that a large proportion of the vapors arising from ripe apples was not acetaldehyde. All subsequent determina-

tions of apple volatiles in this study were made after they had been held at 15 pounds pressure from 6 to 8 hours. The data presented in table 4 indicate that ceric sulfate is sufficiently stable to stand such treatment.

It is realized that ethylene is not appreciably absorbed by concentrated sulfuric acid as used in these determinations.

The apples from which volatile measurements were made were placed in 2-gallon jars in 1941 and in 18-liter jars in 1942. These jars were placed in a constant-temperature bath. The 2-gallon jars held 10 apples and the fruit in them weighed between 1 and 2 kg. In the 18-liter containers anywhere from 40 to 70 apples were used, and these fruits weighed generally about 7 kg. The air passing over the fruit was brought to the desired temperature by having it go through coils of copper tubing, immersed in a water bath at constant temperature before it reached the fruit. The air moved constantly over the apples at the rate of about 28 liters per hour. In 1941 most of the determinations lasted 24 hours, whereas, in 1942, 20-hour runs were made.

TABLE 4.—*Influence of time and temperature on the stability of ceric sulfate*

Oxidation		Ceric sulfate used	Normality of ceric sulfate
Time (hours)	Temperature		
	°C.	*Milliliters*	
8	Room	25	.1200
8	Room	25	.1199
8	Room	25	.1200
8	121	25	.1197
8	121	25	.1197
8	121	25	.1195

Since activated lignite charcoal (Darco) was found to be very effective in absorbing the same vapors from apples that react with sulfuric acid, 50 to 80 gm. of this granulated charcoal was used, in 1941, to cleanse the air before it passed over the experimental fruit. Towers of concentrated sulfuric acid were not used to cleanse the air because it was not possible, with the mercury pump employed, to cause air to flow through two columns of sulfuric acid at once. In 1942, when the weight of the fruit was increased many times, it was found that the air, in a room free of apples and well ventilated with outdoor air, was sufficiently free of material oxidized by ceric sulfate to make special precautions unnecessary. The amount of material found in this air generally amounted to 1 or 2 mg. of ceric sulfate reduced per kilogram of fruit per day. Since the quantities in the air were small and quite constant, they were disregarded. Gerhardt and Ezell (*14*) found only 16 mg. of ceric sulfate to be reduced after collecting the volatiles in fresh air for 6 days at the rate of 10 liters per hour.

The volatile determinations made at 32° and 40° F. throughout the storage season of 1941 and 1942 were taken from 2 bushels of apples placed in air-tight steel drums. The volatile production for each day was measured.

In 1942 aldehyde measurements were made. The aldehydes were removed from the absorption train by placing concentrated solutions of sodium bisulfite in towers similar to those containing the sulfuric

acid. The towers were arranged in series so that the aldehydes reacted with sodium bisulfite before they could be absorbed by the concentrated sulfuric acid. The method used for measuring aldehydes was Tomoda's (*30*) modification of Ripper's procedure. Tomoda's method was modified by making a very strong solution of sodium bisulfite (250–300 gm. per liter). This was essential because the determinations lasted 20 hours and unless a large excess of sodium bisulfite was present at the start of each run, there would be no excess of bisulfite at the end of the measurement. When a large excess of sodium bisulfite was present at the end of a determination, it was removed by passing aldehyde-free air through the tower until only a slight excess remained. This point could be determined by inhaling some of the vapors arising from the tower. When only a faint odor of sulfur dioxide could be detected, a reasonably small excess of sodium bisulfite was present, and one could proceed as outlined by Tomoda (*30*). The concentration of aldehydes from apples harvested in 1942 was found to be so small that 0.01 N iodine or slightly less was used in the titrations. The aldehyde content was calculated as milligrams of acetaldehyde per kilogram of fruit since it is known to be a constituent of apple vapors.

In 1941 the respiration measurements [3] were made on the same days as the volatile determinations, but on similar lots of fruit; in 1942 the volatile and respiration measurements were made on the same lots of fruit. The volatile determinations lasted 20 hours, and the respiration runs were carried out during the remaining 4-hour period.

All the fruits used in these experiments were picked from the orchard at Cornell University, Ithaca, N. Y.

Further details pertaining to individual experiments will be taken up as they are discussed in the text.

RESULTS

INFLUENCE OF STORAGE TEMPERATURES ON RATE OF VOLATILE PRODUCTION

McIntosh apples were picked on September 19, 1941, and two 2-bushel lots of uniform and blemish-free fruits were composited. One lot was placed in a 55-gallon steel drum at 32° F.; the other was placed in a similar container at 40°. Air passed through granular activated charcoal was drawn over the fruit at the rate of 28 liters per hour. The volatiles arising from the apples were measured daily during the storage period for 2 weeks after they were removed from storage, during which time they were held at 74° F. The data for the experiment are presented in figure 2.

Each point in *A* and *B* represents the average daily rate of volatile production for the week previous. The points on the cumulative curves shown in *C* represent the total amount of ceric sulfate reduced per kilogram per week until the apples were removed to 74° F. At 74° the daily production of apple emanations was plotted.

The average rate of volatile production at 40° F. was generally about twice that at 32° throughout the storage season. At 32° the rate of production fell very gradually until January and then rose to a peak in early February. The climacteric in respiration occurred

[3] All respiration measurements were carried out by Dr. R. M. Smock, associate professor of pomology, Cornell University.

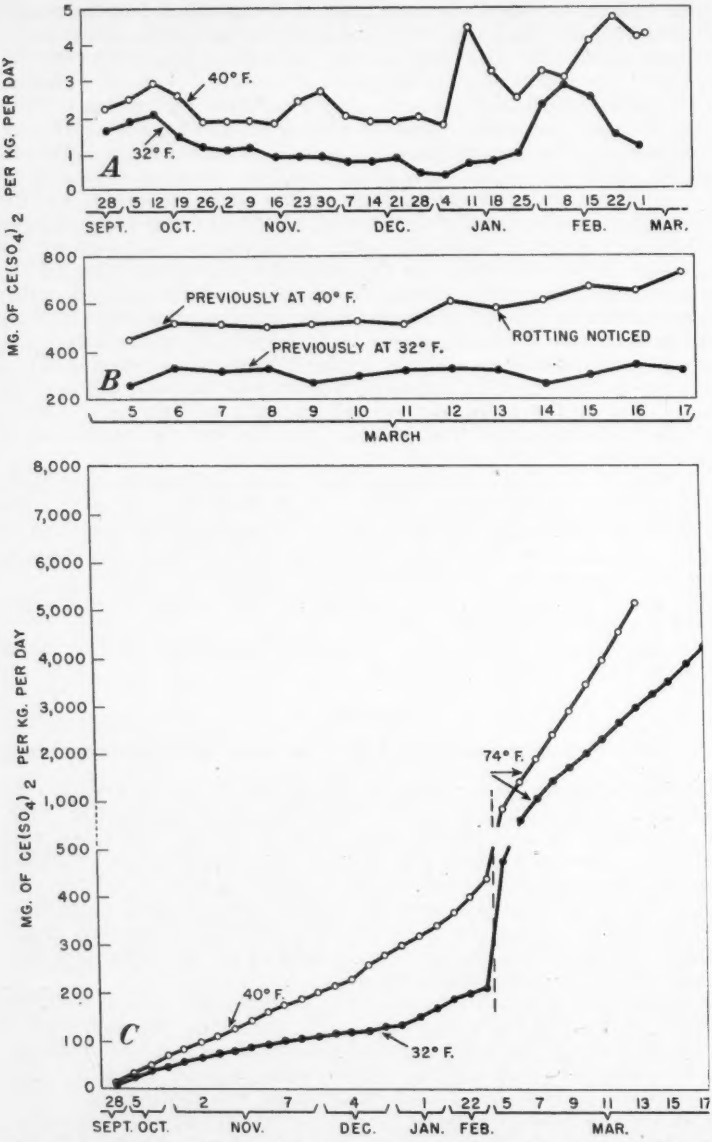
MG. OF $CE(SO_4)_2$ PER KG. PER DAY
40° F.
32° F.
A
28 5 12 19 26 2 9 16 23 30 7 14 21 28 4 11 18 25 1 8 15 22 1
SEPT. OCT. NOV. DEC. JAN. FEB. MAR.
PREVIOUSLY AT 40° F.
ROTTING NOTICED
PREVIOUSLY AT 32° F.
B
5 6 7 8 9 10 11 12 13 14 15 16 17
MARCH
74° F.
40° F.
32° F.
C
28 5 2 7 4 1 22 5 7 9 11 13 15 17
SEPT. OCT. NOV. DEC. JAN. FEB. MAR.

FIGURE 2.—Rate of volatile production from McIntosh apples picked September 19, 1941: *A*, Apples stored at 32° and 40° F.; *B*, apples held at 74° after removal from storage at 32° and 40°; *C*, cumulative curves showing total amount of ceric sulfate reduced per kilogram per week until apples were removed from storage to a temperature of 74°; and thereafter, the daily volatile production until the conclusion of the experiment in March 1942.

in December. The apples held at 40° evolved organic vapors at a rather constant rate until January, at which time the rate rose rather sharply and then continued to increase gradually, except for the period from January 4 to 25, until the fruits were removed from storage in March. The quantity of measurable material arising from the apples at both 32° and 40° was less than 1 percent of the volatile emanations produced by the same fruit at 74°. However, the fruit which had previously been stored at 32° was marketable for a longer period after removal from storage, and as shown by the cumulative curves in figure 2, evolved about as much total volatile material before becoming unmarketable as the apples formerly held at 40°.

INFLUENCE OF MATURITY AND RIPE-APPLE VAPORS ON RATE OF RESPIRATION AND VOLATILE PRODUCTION

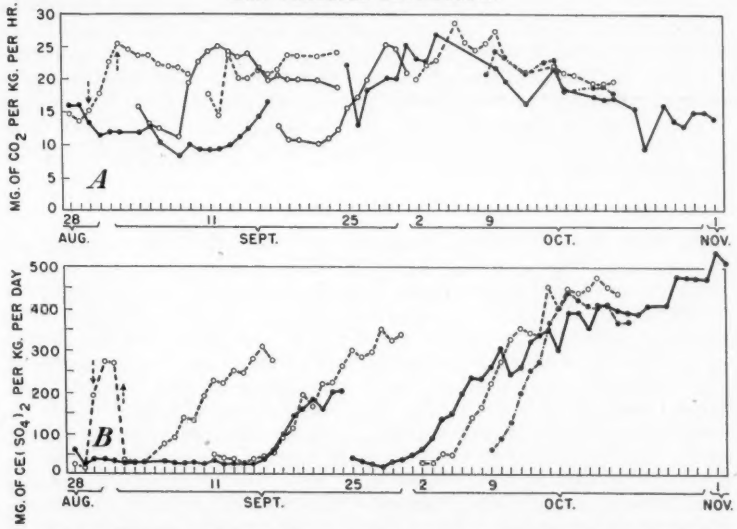

FIGURE 3.—Influence of maturity and ripe apple vapors on rate of respiration and volatile production of McIntosh apples picked at different dates in 1941 and held for different periods at 74° F., as indicated by: *A*, Carbon dioxide produced per kilogram per hour; and *B*, ceric sulfate reduced per kilogram per day. Each curve represents data obtained from apples immediately after they were picked. The broken line, commencing August 28, represents the treated apples, and the solid line the untreated apples. Downward-pointing arrows indicate start of 2-day treatment with vapors from ripe McIntosh apples; upward-pointing arrows, the end of this period.

1941 EXPERIMENT

The data gathered in 1941 showing the influence of maturity on the rate of respiration and volatile production of McIntosh apples are presented in figure 3. There are also some data illustrating the influence of ripe apple vapors on preclimacteric fruit.

The curves for volatile production (fig. 3, *B*) follow the respiratory curves (fig. 3, *A*) quite closely. However, the rise in the rate of production of odorous emanations lags behind the preclimacteric rise in respiration. With the organic vapors there does not seem to be any

sharply defined peak. The amount of volatiles produced continued to rise practically until the experiment ended, although the rate of increase became less pronounced with time. The rate of respiration, on the other hand, rose to a rather well defined maximum and then fell gradually.

In general, it can be stated that the number of days preceding the climacteric rise in respiration and volatile production became less as the date of harvesting was delayed. In fact, the apples picked on October 9 were already respiring at their maximum rate and the odorous emanations reached a climax shortly thereafter.

The influence of vapors from six ripe Early McIntosh apples was tested on one-half bushel of McIntosh apples harvested on August 27. The arrows pointing downward indicate the start of the period during which these ripe apple vapors were drawn over preclimacteric fruit, and the upward-pointing arrows indicate the end of this 2-day treatment. The broken line starting on August 28 represents the treated lot; the solid line represents the control. The volatile measurements during the 2-day period include the vapors from the ripened fruit as well as those from the McIntosh apples. The vapors from the ripe fruit greatly shortened the preclimacteric period of respiration and volatile production.

1942 Experiment

In 1942 the influence of maturity and the effect of vapors from ripened Early McIntosh apples on the rate of respiration and volatile production of McIntosh apples was again studied, but in that year part of the total organic material was measured as acetaldehyde. The data are presented in figures 4 and 5. A comparison of the results in figure 4, *E*, with those in figure 3, *B*, shows that the quantities of ceric sulphate reduced were very much higher in 1941 than in 1942. Since Gerhardt and Ezell (*14*) found that 1 mg. of acetaldehyde reduced about 51 mg. of ceric sulfate, it can be seen that even if the aldehydes are taken into account 15 or 20 mg. of ceric sulfate were reduced per kilogram of fruit per day when the maximum quantities of aldehydes were being produced. The amount of ceric sulfate reduced per kilogram of fruit per day in 1941 rose to between 300 and 450 mg. within 2 weeks after harvest, except for the first picking, whereas in 1942 between 40 and 300 mg. of ceric sulfate were reduced. Only after the last two pickings in 1942 did the quantity of volatile material reduced approach that evolved in 1941 for a similar length of time. The fact that determinations were of 20 hours' duration in 1942 as compared to 24 hours in 1941 would not account for these differences in volatile production. The maximum rates of respiration were also lower in 1942 than in 1941. It was noted that all the varieties studied for apple scald in 1941-42 were injured earlier and much more severely than similar apples in 1942-43.

The shape of the curves for 1942 are quite similar to those for 1941, and the same general conclusions may be drawn from them. Toward the end of the harvesting period the preclimacteric phase became shorter insofar as respiration and volatile production were concerned. Eventually apples were picked (October 9) which were already in their postclimacteric phase of respiration. The increase in the rate of volatile production lagged behind the preclimacteric rise in respiration as it did in 1941.

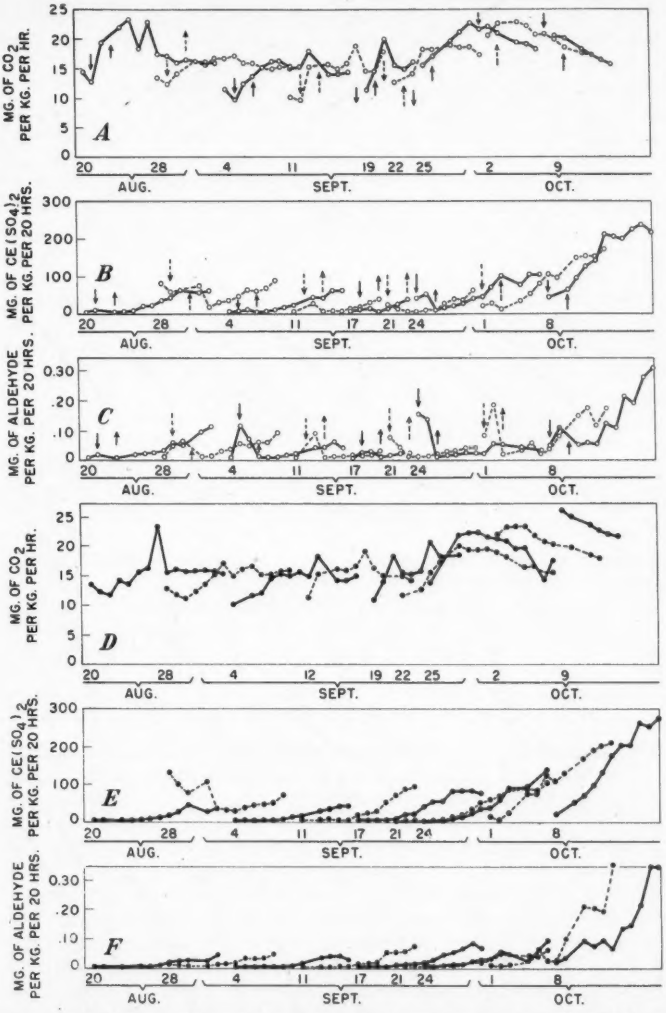
MG. OF CO_2 PER KG. PER HR.
A
MG. OF $CE(SO_4)_2$ PER KG. PER 20 HRS.
B
MG. OF ALDEHYDE PER KG. PER 20 HRS.
C
MG. OF CO_2 PER KG. PER HR.
D
MG. OF $CE(SO_4)_2$ PER KG. PER 20 HRS.
E
MG. OF ALDEHYDE PER KG. PER 20 HRS.
F
20 28 AUG. 4 11 19 22 25 SEPT. 2 9 OCT.
20 28 AUG. 4 11 17 21 24 SEPT. 1 8 OCT.
20 28 AUG. 4 12 19 22 25 SEPT. 2 9 OCT.

FIGURE 4.—Influence of maturity and effect of vapors from ripened Early McIntosh apples on the rate of respiration and volatile production of McIntosh apples, picked at different dates in 1942 and held for different periods at 74° F. *A*, Carbon dioxide given off per kilogram per hour; *B*, ceric sulfate reduced per kilogram in 20 hours; and *C*, aldehyde produced per kilogram in 20 hours. Downward-pointing arrows indicate start of 2-day treatment with vapors from ripened McIntosh apples; upward-pointing arrows, the end of this period. Influence of maturity and effect of vapors from unripened McIntosh apples on the rate of respiration and volatile production of McIntosh apples, picked at different dates in 1942, and held for different periods at 74° F. *D*, Carbon dioxide given off per kilogram per hour; *E*, ceric sulfate reduced per kilogram in 20 hours; and *F*, aldehyde produced per kilogram in 20 hours. Each curve, *A–F*, represents data obtained from apples immediately after they were picked.

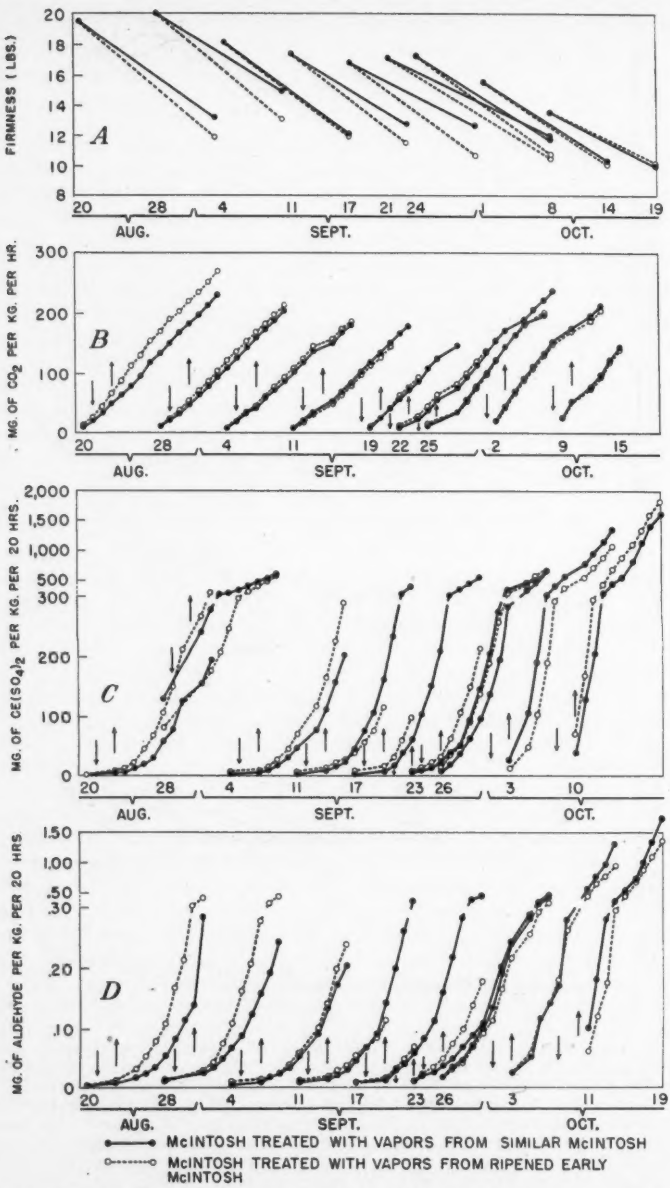

FIGURE 5.—Comparison of firmness, cumulative respiration, and cumulative volatile production from McIntosh apples treated with vapors from ripened and unripened apples at 74° F. *A*, Firmness; *B*, CO_2 given off per kilogram per hour; *C*, ceric sulfate reduced per kilogram in 20 hours; *D*, aldehyde produced per kilogram in 20 hours. Downward-pointing arrows indicate start of 2-day treatment with apple vapors; upward-pointing arrows, end of period.

The graphs in figure 4, *A–C*, represent the rate of respiration and volatile production of McIntosh apples treated with vapors from six ripened Early McIntosh apples for a period of 2 days as indicated by arrows. The graphs in figure 4, *D–F*, show the rate of respiration and volatile production from similar McIntosh apples but treated with vapors from six McIntosh apples harvested at the same time. Figure 5 presents in cumulative form the data given in figure 4 together with the results on the firmness of the flesh at the start and conclusion of each determination.

A careful study of figures 4 and 5 shows that except for the stimulatory effect of the vapors from ripe Early McIntosh apples after the first two pickings (August 20 and 28) and after the sixth picking (September 21), probably no significant effect on respiration or volatile material was produced. It is true that the amount of volatile material produced by McIntosh apples after treatment with vapors from ripened Early McIntosh varies from the production of organic material by similar untreated apples for the last one or two pickings (October 1 and 8), but these differences appear to have been due to natural variations present at the beginning of the experiment rather than to any stimulatory effect of other apple vapors. One fact which seems quite clear is that a stimulatory effect on the rate of respiration or volatile production from ripe-apple vapors occurs when the exposed fruit is in the phase preceding the preclimacteric rise in respiration.

The pressure data are not in complete agreement with the respiration or the volatile data. One would expect that those fruits that respire at a similar rate would become softer at a corresponding rate. This was not always the case. However, those apples that respired at a significantly higher rate were always softer.

RATE OF RESPIRATION AND VOLATILE PRODUCTION FOLLOWING REMOVAL OF APPLES FROM COLD AND MODIFIED ATMOSPHERE STORAGE

An experiment was made in 1942 to determine the rate of respiration and volatile production of McIntosh apples immediately after they were removed from storage. The determinations were made at 74° F. from fruit which had been held previously at 32° in air and at 40° with 5 percent carbon dioxide and 2 percent oxygen until January 2, 1942. The data are presented in figure 6.

The respiration data show that the apples held at 32° F. had already passed through their climacteric rise and were in the postclimacteric phase. The respiratory rate of the apples which came from the controlled atmosphere chamber rose to a peak shortly after they were removed to 74°. These curves indicate that apples respire more slowly after removal from controlled atmosphere storage at 40° than do similar apples after removal from air storage at 32°. The apples stored at 32° were riper, softer, and became unmarketable sooner than the apples held in the controlled atmosphere chamber. Apples brought from a storage room low in oxygen and high in carbon dioxide often seem to lack aroma. The quantitative measurements of volatile emanations show that they are less odorous. Although the rate of respiration gradually decreased during the postclimacteric phase, the rate of volatile production continued to rise even after the apples were unmarketable.

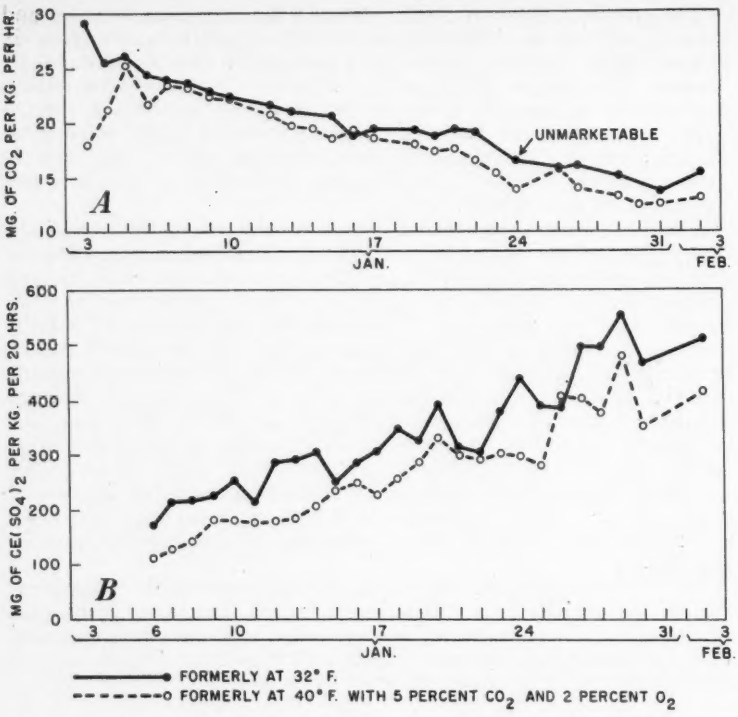

FIGURE 6.—Rate of respiration and volatile production of McIntosh apples at 74° F. after storage at 32° in air and at 40° with 5 percent carbon dioxide and 2 percent oxygen, as shown by: *A*, carbon dioxide produced per kilogram per hour; *B*, ceric sulfate reduced in 20 hours.

DISCUSSION

No one has yet shown which fraction or fractions of apple emanations are related to the scald diseases. Consequently, gross measurements of the apple volatiles reacting with concentrated sulfuric acid might appear to be of little value, for it is conceivable that the quantities of various volatile fractions emanating from apples might vary greatly even though the total volatile production remained constant. Data presented above show that the rate of volatile emission follows the rate of respiration quite closely. These results are similar to those of Kidd and West (*21*) and Gane (*9*, *11*). So far as individual fractions are concerned, the data herein presented show that as those volatiles which are absorbed by sulfuric acid increase, so does the production of aldehydes. Nelson (*23*) has shown that the rate of ethylene emission from McIntosh apples rises as the rate of respiration increases. From the evidence now at hand it seems likely that changes in the rate of production of separate fractions from apples are a result of fluctuations in the rate of respiration, for the peak in the production of these emanations lagged behind the climacteric in respiration. Consequently, comparisons of the total volatiles produced might at

least indicate the relative quantity of scald gases being evolved. The possibility that these differences in apple emanations may be correlated with the time at which scald appears and its severity was noted in 1941–42 and 1942–43. In 1941 the McIntosh apples held at 74° F. evolved a great deal more volatile material than did similar apples in 1942. The scald in 1941 was visible sooner and was much more severe than in 1942. Since the volatile curves resemble the respiratory ones, it might be possible to predict the severity of scald by comparing the carbon dioxide measurements from year to year during the fall. Although there were differences in respiration between 1941 and 1942, they were relatively small when compared to the variations in volatile production between these 2 years.

SUMMARY AND CONCLUSIONS

This paper reports the results of a study to determine the influence of various factors on the production of volatile material from apples and on the rate of their respiration.

McIntosh apples stored at 32° F. were found to evolve about half as much volatile material as similar apples held at 40°. When the fruits were removed from storage and placed at 74°, they emitted more organic vapors in 1 day than they had during 5 months in storage. Apples which had previously been held at 40° continued to evolve more volatile material than fruits which were formerly held at 32°.

Apples stored in an atmosphere of 5 percent carbon dioxide and 2 percent oxygen at 40° F. respired and produced volatiles at a slower rate, when removed to room temperature, than similar fruit previously stored at 32° in air.

The quantity of volatile material arising from McIntosh apples harvested in 1941 and held at 74° F. was found to be much higher than that from similar fruit harvested in 1942. As the severity of scald was much more pronounced in 1941–42 than in 1942–43, it has been suggested that differences in apple emanations from one year to the next may be correlated with the amount of apple scald that will occur during the storage season for any given stage of maturity and set of storage conditions.

Apples which are in the preclimacteric stage may be stimulated by an active agent (ethylene) so that the rate of volatile production as well as respiration is increased.

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THE REMOVAL OF ORGANIC EMANATIONS FROM THE ATMOSPHERE SURROUNDING STORED APPLES[1]

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INTRODUCTION

The work of Brooks and his associates (*1, 2, 3, 4, 5*)[2] indicated that the cause of apple scald is closely related to the organic emanations arising from the apples themselves. Smock and Southwick (*22*) further found that one lot of apples may hasten the development of scald on another lot in storage, and Smock (*21*) showed that ethylene arising from postclimacteric apples may shorten the storage life of preclimacteric apples by as much as 25 percent.

Since both rate of ripening and scald development appear to be related to the presence of organic apple emanations, an atmospheric washing agent was sought which would remove the emanations and so improve the quality and lengthen the life of stored applies. Oiled paper used as wraps about the fruit or shredded and mixed with them is the most practicable method of controlling scald at the present time. However, in the northeastern apple-growing sections this method has often given unsatisfactory control. The use of oiled paper also involves considerable labor and expense. Consequently, a more effective and simpler way of obtaining scald control is needed.

To be of practical value to commercial storage operators any material employed to "air-condition" a storage atmosphere should be inexpensive, readily obtainable, easy to use, and harmless to the fruit. Therefore, emphasis has been placed on some compounds which probably could be used commercially, if found satisfactory from the standpoint of their ability to remove volatile materials.

Measurements have been made of the absorptive and adsorptive capacity of various materials for vapors of ethyl acetate, those apple emanations which react with concentrated sulfuric acid, and the ethylene in ripe apple vapors. Respiration measurements were made in conjunction with most of the volatile measurements.[3]

REVIEW OF LITERATURE

An analysis of apple emanations by Power and Chesnut (*16, 17*) showed the presence of amyl esters of formic, acetic, caproic, and caprylic acids, ethyl alcohol, acetaldehyde, and geraniol. Ethylene has been identified, also, as a constituent of apple emanations by many other investigators (*6, 8, 14, 15*).

[1] Received for publication January 4, 1944.

[2] Italic numbers in parentheses refer to Literature Cited, p. 313.

[3] These measurements were made by Dr. R. M. Smock, associate professor of pomology at Cornell University, and have already appeared in published form (*21*). They are presented here, with his permission, merely to show the relationship between them and the rate of volatile production.

A brief review of the methods employed to measure organic vapors arising from fruits has been given by Gerhardt and Ezell (*11*).

Combustion techniques as employed by Gane (*7*, *9*) and Kidd and West (*14*) offer one of the most suitable laboratory methods of removing all the organic materials arising from fruits if the rate of air flow is slow.

Concentrated sulfuric acid (specific gravity, 1.84) was suggested by Gerhardt and Ezell (*11*) as a suitable oxidizing agent for all the known organic emanations arising from apples. Later, Gerhardt (10) recognized that concentrated sulfuric acid was ineffective as an ethylene absorbent in his previous experiments. However, Tropsch and Mattox (*25*) found that sulfuric acid treated with nickel and silver sulfates was able to remove ethylene from gaseous hydrocarbons much more efficiently than untreated acid. Consequently, Gerhardt (*10*) assumed that ethylene was absorbed from a stream of air containing apple emanations when the vapors were passed through 10 ml. of an activated acid mixture (1 part concentrated sulfuric acid saturated with nickel sulfate at room temperature to 15 parts of acid possessing 0.6 percent of silver sulfate at room temperature).

Walls (*26*), using an air flow of 3 liters per hour, found that 90 percent of the ethylene was removed from air containing 1 part of ethylene per 10,000 of air, if 0.8 gm. of silver sulfate was added to 40 ml. of concentrated sulfuric acid. He found silica gel and activated carbon (Desorex III) ineffective at room temperature in adsorbing ethylene The materials were suitable, however, when cooled to minus 40° C.

Smith and Gane (*18*) were able to remove the ripening agent (ethylene) from apple vapors by the use of bromine, ozone, fuming nitric, and fuming sulfuric acid.

It is obvious that those materials which are most effective in removing apple vapors from the atmosphere are unsuitable for commercial use, with the possible exception of ozone. The evidence obtained from the use of ozone either as a scald control measure (*3*) or as an oxidizing agent for apple emanations (*18*) is inconclusive at this time.

METHODS

The experiments were carried out in 1942 with apples collected from the Cornell University orchard at Ithaca, N. Y. Blemish-free fruits were selected, and composited, and placed in 18-liter jars. Special precautions were taken so that none of the experimental apples were exposed to vapors from ripe apples prior to the experimental period.

The jars containing the fruit were placed in a constant temperature water bath where the temperature was maintained at 74° F., unless otherwise stated. A mercury pump was employed as a means of supplying air movement around the fruit. The air passing over the apples was brought to that of the bath by first being drawn through copper coils immersed in the bath. The absorption period of volatile emanations from the apples generally lasted 20 hours. The respiration measurements were made during the subsequent 4-hour period on the same lot of fruit.

PROCEDURES EMPLOYED FOR MEASURING ORGANIC APPLE EMANATIONS AND OIL VAPORS

Absorption by sulfuric acid.—The measurements of organic apple, oil, and ethyl acetate vapors were made by using a method similar to one described by Gerhardt and Ezell (*11*). This method was modified somewhat by employing higher temperatures when oxidizing the vapors absorbed in concentrated sulfuric acid (specific gravity, 1.84). The reasons for this modification have already been discussed (*23*). Briefly, this procedure consists of absorbing apple emanations, from a stream of air, in towers containing 35 ml. of concentrated sulfuric acid and then oxidizing them with known volumes of a standard solution of ceric sulfate (approximately 0.1 N) in at least a 1 molar concentration of acid. The oxidation process was carried out at 15 pounds' steam pressure (approximately 121° C.) for at least 6 hours. The excess ceric sulfate was titrated with 0.1 N ferrous sulfate; orthophenanthroline ferrous sulfate was used as the indicator (*19*). The results are expressed as milligrams of ceric sulfate reduced per kilogram of fruit per 20 or 24 hours. This method is not suitable for ethylene absorption, however, since ethylene is not readily absorbed in concentrated sulfuric acid (*10*).

Absorption by sodium bisulfite.—Aldehyde determinations were made by Tomoda's method (*24*). The measurements were obtained by placing towers containing 40 ml. of a solution of concentrated sodium bisulfite in series with the tower containing the concentrated sulfuric acid. These towers were so arranged that the apple vapors originally passed through the sodium bisulfite and, consequently, only aldehyde-free vapors reached the tower containing the sulfuric acid. Since acetaldehyde is a known constituent of apple vapors (*16*), the aldehydes were calculated as milligrams of acetaldehyde per kilogram of fruit per 20 or 24 hours. Further details concerning the method have appeared elsewhere (*23*).

MATERIALS EMPLOYED FOR ABSORBING ETHYL ACETATE AND APPLE VAPORS

Oils.—An attempt was made to determine how effective certain oils (products of the Shell Company) would be in absorbing the ester, ethyl acetate, since it has been thought by some (*3*) that esters are related to the production of apple scald. These determinations were made by passing known weights of ethyl acetate vapors through 25-ml. samples of oil at the rate of 28 to 36 liters per hour. Any ethyl acetate which was not removed by the oils was taken up by a tower of sulfuric acid and the milligrams of ceric sulfate reduced were calculated. The apparatus used has been described in a previous paper (*23*).

Further determinations were made with oils to ascertain their capacity for removing apple emanations. In these experiments lots of McIntosh apples were placed in a temperature-control bath at 74° F. The quantity of organic material arising from the fruit was determined for a few days before samples of the oils were inserted in the series between the fruit and the concentrated acid. By following this procedure it was hoped that the percent of the total apple vapors absorbed by the oils could be determined.

Activated charcoal.—Since activated charcoals are frequently used for adsorbing organic gases and impurities from liquids, a number of tests were made with an activated lignite charcoal (Darco) on the re-

moval of ethyl acetate and organic apple emanations. Samples of both powdered and granular (4 by 12 mesh) were employed. The ability of the charcoal to adsorb ethyl acetate and organic apple vapors was determined at room temperature by following procedures similar to those outlined under the discussion of methods of testing oils. Other details relating to the use of charcoal are presented with the results.

MATERIALS EMPLOYED FOR REMOVING ETHYLENE FROM APPLE VAPORS

Kidd and West (*13*) and Smock (*20*) have shown that ripe apple vapors will stimulate the respiratory rate of preclimacteric apples. Since ethylene has a similar effect on immature apples it has been assumed that ripe apples liberate sufficient ethylene to hasten the rate of ripening of immature fruit. Therefore, the adsorbents or absorbents tested to remove this ripening agent were placed in series between post- and pre-climacteric fruit. The preclimacteric apples were held at 74° F., unless otherwise stated, whereas the postclimacteric fruit and the materials to be tested were kept at room temperature. Air was drawn at the rate of 28 liters per hour from six ripened apples over half-bushel lots of preclimacteric fruit for 2 days as indicated on the figures. Vapors from six apples of the same variety and maturity were drawn over the control lot. Daily measurements were made of the rate of respiration and volatile production of the test fruit. By observing the changes in the rate of respiration, volatile production, and firmness of flesh one can determine which materials have removed ethylene from the ripe apple vapors. At least it will be possible to tell which materials have eliminated ehtylene to a point where it no longer hastens ripening of immature apples.

Oils.—The oils used were obtained from the Sun Oil Co. Fifty-milliliter samples of the oils were placed in towers 24 inches high and 1 inch in diameter. These columns were filled with tightly packed steel wool so that a large surface of oil would be exposed to the ripe apple vapors as they passed through the absorption towers. Oil D3 was napthenic while oils I1 and A5 were paraffinic in nature.

Activated charcoal.—Samples of granular, activated, lignite charcoal (4 by 12 mesh) were placed in towers similar to those used for the oils.

Brominated, activated charcoal.—Samples of granular, activated, lignite charcoal were saturated with bromine. These samples were then aerated in an effort to remove as much of the "free" bromine vapors from the charcoal as possible. However, special precautions, as discussed later, were necessary to remove the bromine vapors which arose from the towers.

Alkaline potassium permanganate.—The solution used consisted of 50 milliliters of water saturated with potassium hydroxide combined with 50 milliliters of water saturated with potassium permanganate. The absorption tower employed was similar to the one used for the oils.

Activated sulfuric acid.—Thirty-five milliliters of this material were prepared as directed by Tropsch and Mattox (*25*). The solution consists of 1 part concentrated sulfuric acid (specific gravity, 1.84) saturated with nickel-sulfate at room temperature to 15 parts of acid possessing 0.6 percent of silver sulfate. The solution was placed in an absorption tower containing solid glass beads.

RESULTS

MEASUREMENTS OF THE VOLATILE PRODUCTS LIBERATED BY SOME OILS

Before the value of the oils as absorbents of some organic emanations could be determined, it was necessary to find the quantity of volatile material released by the oils themselves.

The results are given in table 1. Spuria 22 and Latus 29 were oils of low refinement, Aurita 22 was of medium refinement, and Oliva 34 was of high refinement. Oliva 34 was also a nonoxidizing oil. There is a definite correlation between the refinement of an oil and the total volatile material produced. Oils of low refinement produce the greatest amount of volatile substances. The refinement of the Protego oil was unknown. In general, less volatile material was released by these oils with time.

TABLE 1.—*Volatiles liberated from 25-ml. samples of different oils*

Oil type	Ceric sulfate reduced per day by sample No. —					Total
	1	2	3	4	5	
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
Spuria 22	207.8	424.3	292.3	150.7	135.6	1,210.7
Latus 29	57.9	101.9	92.0	89.2	59.5	400.5
Aurita 22	60.7	71.8	61.9	35.7	40.8	270.9
Oliva 34	38.5	35.7	49.2	30.1	35.2	188.7
Protego	104.6	90.7	73.0	13.9	35.2	317.4
Air	25.0	34.5	31.8	8.4	8.4	108.1

ABILITY OF CERTAIN OILS AND ACTIVATED CHARCOAL TO REMOVE VAPORS OF ETHYL ACETATE AND SOME APPLE EMANATIONS

OILS

The effectiveness of oils in absorbing vapors of ethyl acetate was determined by passing known weights of ethyl acetate vapors through 25-ml. samples of oil.[4] Any ethyl acetate which was not removed by the oil was taken up by a tower of sulfuric acid and the milligrams of ceric sulfate reduced were calculated. Since the oils themselves liberate volatile material, it was realized that some of the ceric sulfate was reduced by the emanations from the oils as well as from the ethyl acetate which passed unabsorbed through the oil. However, these measurements lasted only 1 hour per day so that the amount of volatile material arising from the most unrefined oils probably did not exceed 20 mg. of ceric sulfate reduced. From the results shown in table 2, where quantities from 845 to 3,500 mg. of ceric sulfate were reduced, it can be seen that even 20 mg. of ceric sulfate reduced by the oil volatiles would be insignificant. The reduction-oxidation ratios,[5] the milligrams of ethyl acetate absorbed, and the percent absorption, were calculated. It was assumed that all the ceric sulfate reduced was due to the ethyl acetate alone. The milligrams of ethyl acetate absorbed and the percent absorption were calculated assuming the average reduction-oxidation value to be 28.27 as shown by Southwick (*23*).

[4] The apparatus used has been described (*23*).
[5] A term employed by Gerhardt and Ezell (*11*).

TABLE 2.—*The absorptive capacity of some oils for ethyl acetate*

Oil type (25 ml.)	Ethyl acetate	Ceric sulfate reduced	Reduction-oxidation ratio	Ethyl acetate absorbed	Degree of absorption
First day:	*Milligrams*	*Milligrams*		*Milligrams*	*Percent*
Spuria 22	87.9	1,517.2	17.26	34.2	38.9
Latus 29	61.7	845.8	13.71	31.8	51.5
Aurita 22	76.9	1,433.2	18.64	26.2	34.1
Aurita 32	84.0	1,618.7	19.27	26.7	31.8
Oliva 27	74.3	1,529.0	20.58	20.2	27.2
Oliva 34	79.8	1,484.4	18.60	27.3	34.2
Protego	79.1	1,592.4	20.13	22.8	28.8
Second day:					
Spuria 22	99.6	2,828.9	28.40	−.5	−.5
Latus 29	79.4	1,942.9	24.47	10.7	13.5
Aurita 22	78.2	2,174.5	27.81	1.3	1.7
Aurita 32	71.3	1,903.9	26.70	4.0	5.6
Oliva 27	65.5	1,788.8	27.31	2.2	3.4
Oliva 34	91.9	2,638.7	28.71	−1.4	−1.5
Protego	153.6	3,540.2	23.05	28.4	18.5

One difficulty in making direct comparisons of one oil with another is that the quantities of ethyl acetate passing through each oil were not identical. The data do indicate that the most unrefined oils, Spuria 22, Latus 29, and Protego, are somewhat superior to the highly refined oils so far as their capacities for absorbing ethyl acetate is concerned. None of the oils appear to be very effective, however. It is suggested that the reason the sample of Protego seems so much superior to the other oils on the second day may be due to the larger concentration of ethyl acetate passing through it during the absorption period. A higher vapor pressure of ethyl acetate in this case may well have resulted in a greater absorption of this ester in the oil.

Further determinations were made with a number of oils. In these experiments lots of McIntosh apples were placed in a temperature control bath at 74° F. The quantity of organic material absorbed in surfuric acid and the quantity of ceric sulfate reduced were recorded by the usual procedure. Then towers containing 25-ml. samples of oil were inserted in the series between the fruit and the concentrated acid. It is apparent that the oils removed some of the volatile material since the amount of ceric sulfate reduced was higher before and after the oils were removed from the series (table 3). Although the apples were held at a constant temperature of 74° F., the rate of volatile production was not constant from day to day. Consequently, the actual amount of volatile material removed by the oils could not be calculated. It was though tthat perhaps the quantity of material absorbed by the oils could be measured by aerating them after they had been subjected to apple emanations. This was done on January 19 and January 20 as indicated in table 3. The results show that there was more volatile material arising from these oils after the first day of aeration than after the second day. Since the quantities of organic vapors emitted from the oils themselves, as shown in table 1, were not constant, it cannot be assumed that the difference in volatile production from January 19 to January 20 (table 3) represents only the apple emanations which had been removed from the oils. However, by comparing the loss in organic vapors from oils which had been aerated for 5 days (table 1) with the losses of the same oils in this experiment after an equal number of days, one can see that the amounts of ceric sulfate reduced are quite similar.

TABLE 3.—*The ability of oils to absorb vapors from 10 McIntosh apples at 74° F*

Date	Treatment	Ceric sulfate reduced per day by—					
		Aurita 22	Latus 29	Spuria 22	Oliva 34	Protego	No oil
		Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
Jan. 11	Apples alone	648.3	632.6	650.7	688.8	512.7	680.8
12	do	493.2	672.3	389.3	697.8	389.3	624.1
13	do	498.4	334.4	585.2	732.1	301.0	722.4
14	do	739.2	692.7	548.4	712.5	444.9	528.6
15	Oils added	481.8	458.7	488.5	651.5	308.8	541.6
16	do	463.5	476.6	403.2	546.8	306.9	503.5
17	do	435.8	452.4	409.6	521.8	297.4	549.1
18	Apples alone	680.4	703.4	561.4	749.4	459.4	759.3
19	Oils only	213.3	115.0	269.7	154.2	49.2	[1] 13.1
20	do	42.4	58.6	111.1	26.2	26.2	[1] 9.9

[1] Air.

ACTIVATED CHARCOAL

The adsorptive capacity of 25 gm. of powdered, activated lignite charcoal (Darco) for ethyl acetate was measured by employing the same procedure and air flow as was used with the oils, except that the length of time per treatment lasted from 1 to 8 hours. The data are presented in table 4. A comparison of the data in the last column of tables 2 and 4 shows the charcoal to be vastly superior to the oils insofar as its capacity to remove this ester is concerned. The charcoal was practically a perfect adsorbent for the first 1.8 gm. of ethyl acetate which passed through it. The adsorptive capacity of the charcoal dropped markedly during the last treatment after approximately 2.6 gm. had been absorbed.

In an effort to determine how effective this activated charcoal would be in adsorbing the volatile materials arising from apples which react with sulfuric acid, an 80 gm. sample of charcoal was tested. This sample of granular, activated charcoal was selected because it had been used previously to absorb apple volatiles for 5 months from a bushel of Cortland and a bushel of McIntosh apples held at 40° F. The author was anxious to know whether this sample of charcoal had become saturated with adsorbed apple vapors during the 5-month period. The emanations from 10 ripe Cortland apples at room temperature were absorbed in sulfuric acid and the quantity of material produced on April 4 and 5 was determined in terms of milligrams of the sulfate reduced. On April 6 and 7 the charcoal was inserted, as an adsorbent, in series between the apples and on April 8 and 9 the concentrated acid was removed. The results are given in figure 1.

TABLE 4.—*The adsorptive capacity of 25 gm. of powdered activated charcoal for ethyl acetate*

Treatment No.	Ethyl acetate	Ceric sulfate reduced	Reduction-oxidation ratio	Ethyl acetate adsorbed	Degree of adsorption
	Milligrams	*Milligrams*		*Milligrams*	*Percent*
1	88.4	45.8	0.52	86.8	98.19
2	228.7	27.5	.12	227.7	99.56
3	627.8	7.6	.01	627.5	99.95
4	861.0	6.4	.01	860.8	99.98
5	796.9	5,099.5	6.40	616.5	77.36
6	79.2	1,872.5	23.64	13.0	16.41

This same sample of charcoal was also subjected to vapors from one-half bushel of ripe McIntosh apples on April 8 and 9. The daily rate of volatile production of the McIntosh apples was determined for 3 days (April 5, 6, and 7) before the activated charcoal was tested. On April 10 no material was used in removing apple vapors, but on April 11 a 25-ml. sample of fresh Protego oil was employed as an absorbent. No adsorbent was used on April 12. The comparative abilities of the activated charcoal and Protego oil

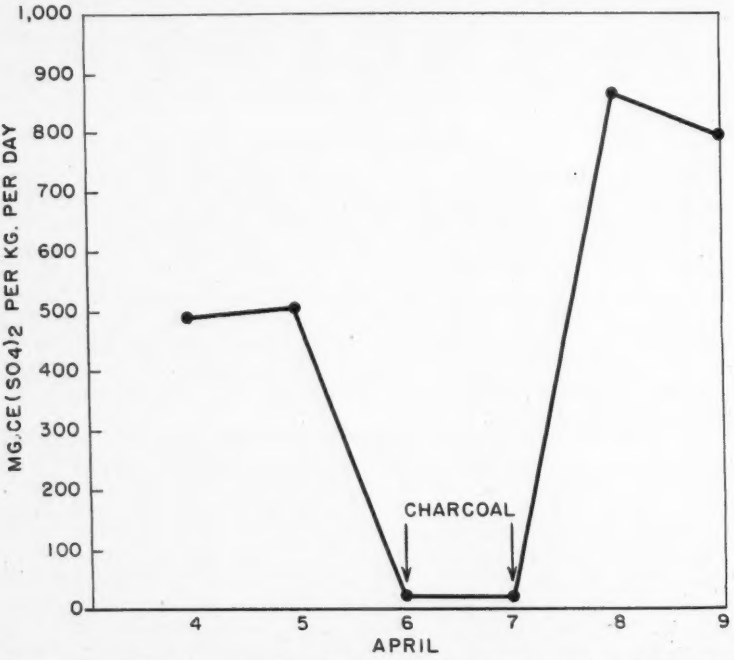

FIGURE 1.—Adsorptive capacity of activated charcoal for apple volatiles that react with concentrated sulfuric acid, as indicated by experiments conducted on Cortland apples at room temperature. Arrows indicate when charcoal was used to adsorb apple emanations.

to remove aldehydes and organic apple emanations is shown in figure 2. It is apparent that granular, activated lignite charcoal is an excellent adsorbent for apple vapors that react with concentrated sulfuric acid and that Protego oil is very much inferior to it. It is also apparent that the charcoal was not saturated with the apple vapor arising from apples held for 5 months at 40° F.

VALUE OF DIFFERENT MATERIALS FOR REMOVING THE RIPENING AGENT (ETHYLENE) FROM APPLE EMANATIONS

Although the results shown in figures 1 and 2 demonstrate that activated lignite charcoal is effective in removing apple vapors that react with concentrated sulfuric acid, the problem of determining which materials remove ethylene has yet to be solved.

In the experiments described below the value of several oils, activated charcoal, brominated, activated charcoal, alkaline potassium permanganate, and activated sulfuric acid as ethylene removers was determined. Preclimacteric Wealthy, McIntosh, and Rhode Island Greening apples were selected and held at 74° F. (except in the experiment noted in figure 3, where the temperature was maintained at 78° F.). Vapors from six ripened apples were passed over half-bushel lots of unripe fruit for 2 days, as indicated by arrows in figures 3, 4,

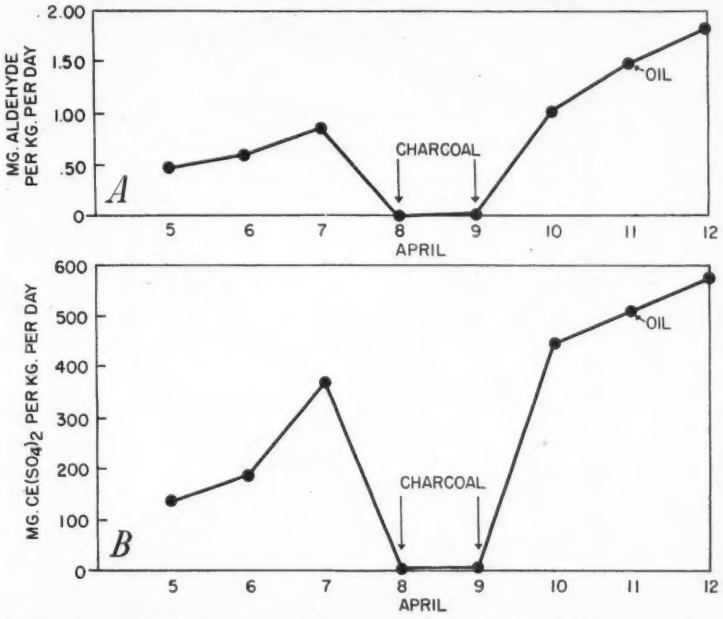

FIGURE 2.—Adsorptive and absorptive capacity of activated charcoal and oil, respectively, for apple volatiles that react with concentrated sulfuric acid, as shown by experiments conducted at room temperature on: *A*, Cortland apples; *B*, McIntosh apples. Arrows indicate when charcoal and oil were used to remove apple emanations.

5, 6, and 7. Vapors from six apples of the same variety and stage of maturity were drawn over the control lot at the same time.

In the first of this series of experiments (figs. 3) three oils (products of the Sun Oil Co.) were studied. None of the 50-ml. samples of these oils was capable of lowering the concentration of the ripening agent contained in ripe Yellow Transparent apple vapors below its stimulatory level. This conclusion is substantiated by the volatile measurements as well as by the respiratory determinations. The control lot was also firmer than the other groups of fruit. However, the apples which had been subjected to Yellow Transparent apple emanations, in the absence of oil as an absorbent, were softest.

Figure 4 shows the results of an experiment where 100 ml. of alkaline potassium permanganate, 80 gm. of granular, activated charcoal, and

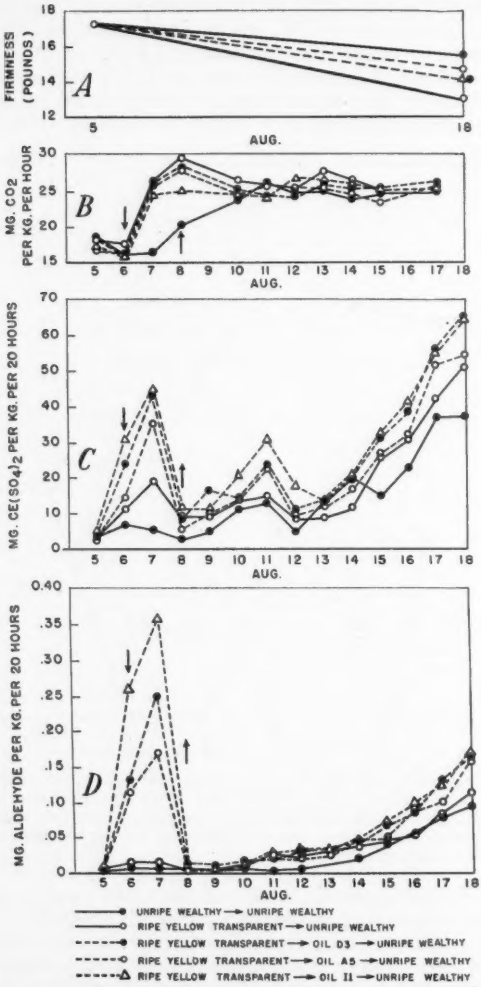
FIRMNESS (POUNDS)
A
AUG.
MG. CO2 PER KG. PER HOUR
B
AUG.
MG. CE(SO4)2 PER KG. PER 20 HOURS
C
AUG.
MG. ALDEHYDE PER KG. PER 20 HOURS
D
AUG.
UNRIPE WEALTHY → UNRIPE WEALTHY
RIPE YELLOW TRANSPARENT → UNRIPE WEALTHY
RIPE YELLOW TRANSPARENT → OIL D3 → UNRIPE WEALTHY
RIPE YELLOW TRANSPARENT → OIL A5 → UNRIPE WEALTHY
RIPE YELLOW TRANSPARENT → OIL II → UNRIPE WEALTHY

FIGURE 3.—A comparison of the firmness, respiration, and volatile production of unripe Wealthy apples treated with vapors from ripe Yellow Transparent at a temperature of 78° F., and of the ability of three commercial oils to remove a ripening agent (ethylene) from these vapors. *A*, Firmness; *B*, carbon dioxide given off per kilogram per hour; *C*, ceric sulfate reduced per kilogram in 20 hours; *D*, aldehyde produced per kilogram in 20 hours. Downward-pointing arrows indicate start of 2-day treatment with apple vapors; upward-pointing arrows, the end of this treatment.

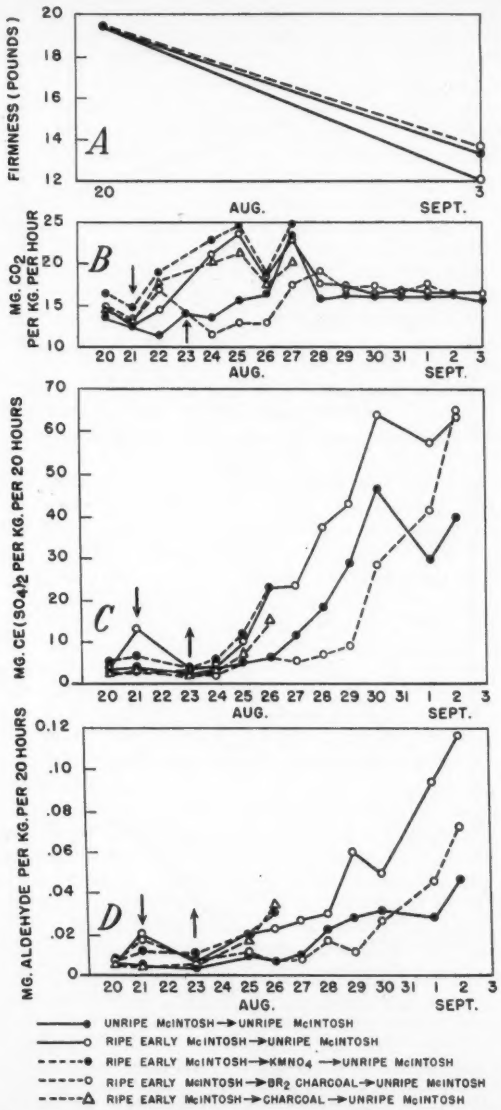

FIGURE 4.—A comparison of the firmness, respiration, and volatile production of unripe McIntosh apples, treated with vapors from ripe Early McIntosh apples at a temperature of 74° F., and of the ability of alkaline potassium permanganate, activated charcoal, and brominated, activated charcoal to remove a ripening agent (ethylene) from these vapors. *A*, Firmness; *B*, carbon dioxide given off per kilogram per hour; *C*, ceric sulfate reduced per kilogram in 20 hours; *D*, aldehyde produced per kilogram in 20 hours. Downward-pointing arrows indicate start of 2-day treatment with apple vapors; upward-pointing arrows, the end of this treatment.

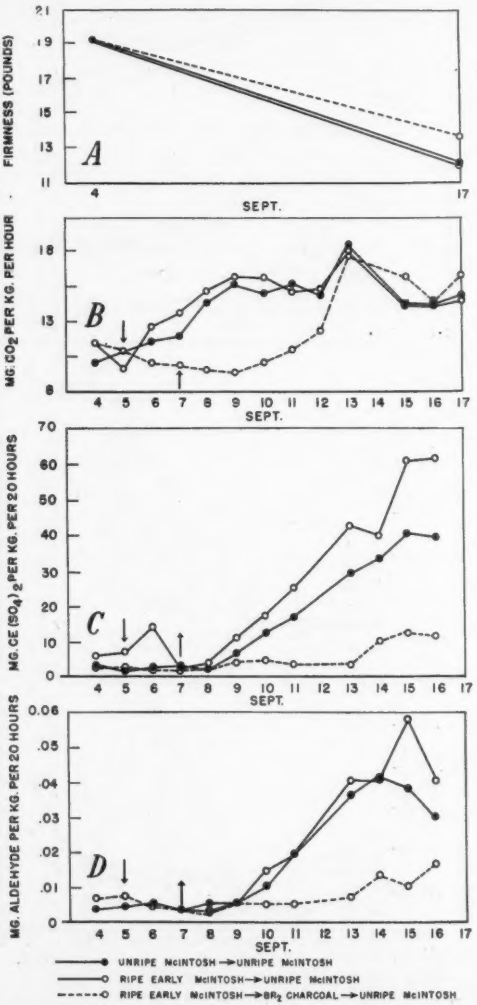

FIGURE 5.—A comparison of the firmness, respiration, and volatile production of unripe McIntosh apples treated with vapors from ripe early McIntosh apples at a temperature of 74° F., and of the ability of brominated, activated charcoal to remove a ripening agent (ethylene) from these vapors. *A*, Firmness; *B*, carbon dioxide given off per kilogram per hour; *C*, ceric sulfate reduced per kilogram in 20 hours; *D*, aldehyde produced per kilogram in 20 hours. Downward-pointing arrows indicate start of 2-day treatment with apple vapors; upward-pointing arrows, the end of this treatment.

the same weight and type of charcoal saturated with bromine were used to remove ethylene from ripe early McIntosh apple emanations.

In all instances except those in which the brominated charcoal was employed the ripe apple emanations stimulated an early climacteric rise in respiration and volatile production as compared to the check. However, some of the bromine from the brominated charcoal passed over this experimental lot of apples. This was evident from the fact that a solution of sodium hydroxide placed in the series for a few hours became light yellow as the bromine vapors reacted with it. As a consequence, one cannot be certain whether the bromine held on the charcoal or the bromine in the air stream was responsible for the removal of the active agent in the Early McIntosh vapors. The apples in the group in which the brominated charcoal was employed were as firm as the control lot at the end of the experiment.

The results recorded in figure 5 substantiate those shown in figure 4. In this experiment the presence of brominated charcoal appeared to have retarded the initiation of the climacteric rise in respiration and volatile production. The control lot produced volatiles and carbon dioxide at a rate comparable to that of the apples treated with ripe apple vapors in the absence of an adsorbent. The marked delay in the development of the climacteric rise in respiration and volatile production of those McIntosh apples with which brominated charcoal was used may have been due to the presence of bromine on the surface of the apples. In this experiment, as in the previous one (fig. 4), some bromine vapor was carried over to the experimental fruit in the air stream even though a sodium hydroxide gas washing solution was employed. The presence of free bromine on the surface of these apples might have reacted with any ethylene produced by the apples themselves, and so have delayed the climacteric rise in respiration and volatile production.

An effort was made in the experiment presented in figure 6 to determine whether the ability of brominated charcoal to remove the active agent in ripe apple vapors was due to the bromine vapors arising from the brominated charcoal or to the bromine actually adsorbed on the charcoal. Forty grams of the saturated bromine charcoal were mixed with an equal part of unbrominated, activated charcoal. By such a procedure any excesses of loosely held bromine were adsorbed by the unbrominated fraction. After this mixture was prepared and well stirred no bromine could be detected by the sense of smell or by the appearance of a light yellow color when air from this charcoal was passed through a solution of sodium hydroxide. This 80-gm. sample was placed in a tower between the six ripe McIntosh and the half bushel of preclimacteric Rhode Island Greening apples. A 40-gm. sample of the saturated bromine charcoal was placed before the ripe McIntosh apples, as shown in figure 6, so that the bromine vapors arising from this charcoal passed over the ripe fruit as well as over the preclimacteric Rhode Island Greening apples.

The results show that brominated charcoal may remove ethylene from ripe apple vapors even in the absence of "free" bromine vapors.

An attempt was made to determine whether 35 ml. of activated sulfuric acid, prepared as directed by Tropsch and Mattox (*25*), would remove ethylene from apple vapors below a stimulatory concentration. Vapors from ripe Rhode Island Greening apples were

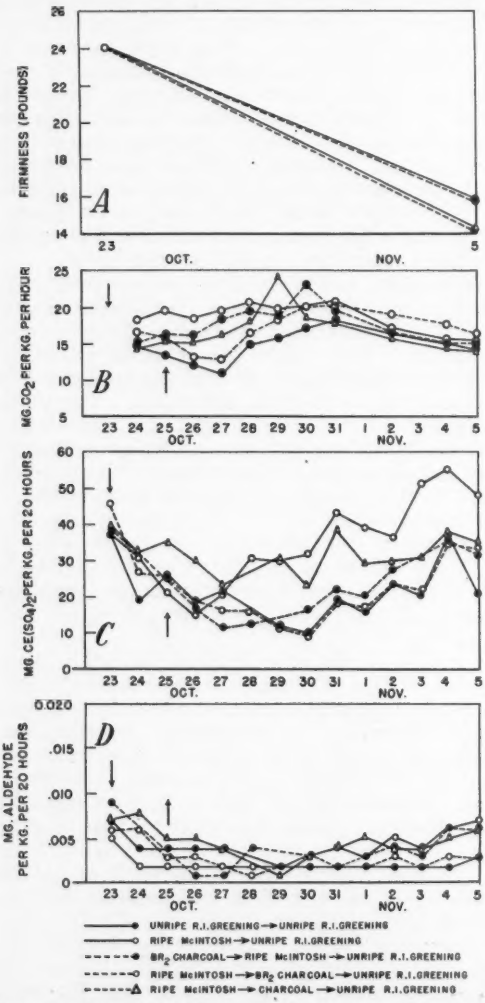

FIGURE 6.—A comparison of the firmness, respiration, and volatile production of unripe Rhode Island Greening apples treated with vapors from ripe McIntosh apples at a temperature of 74° F., and of the ability of activated charcoal, brominated, activated charcoal, and the vapors from brominated activated charcoal to remove a ripening agent (ethylene) from these vapors. *A*, Firmness; *B*, carbon dioxide given off per kilogram per hour; *C*, ceric sulfate reduced per kilogram in 20 hours; *D*, aldehyde produced per kilogram in 20 hours. Downward-pointing arrows indicate start of 2-day treatment with apple vapors; upward-pointing arrows, the end of this treatment.

drawn over preclimacteric apples of the same variety after these ripe apple emanations had passed through the adsorption tower containing the activated acid. The results shown in figure 7 indicate that the

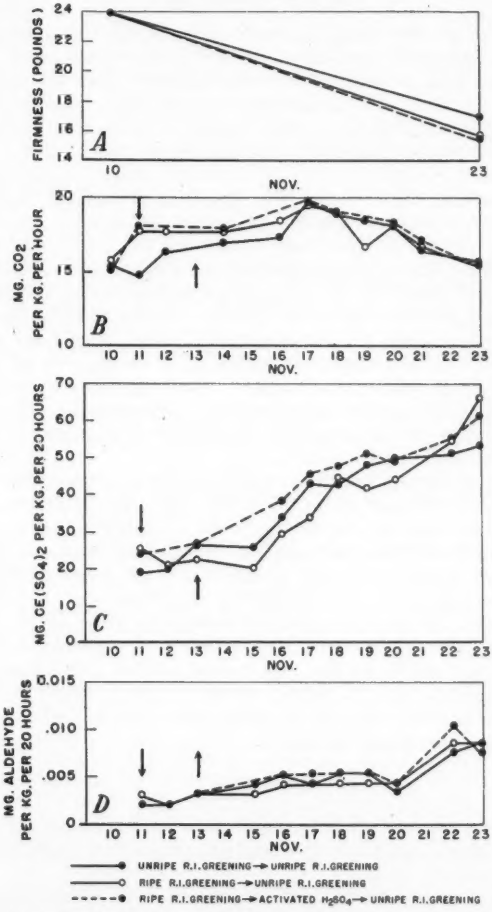

FIGURE 7.—A comparison of the firmness, respiration, and volatile production of unripe Rhode Island Greening apples treated with vapors from ripe Rhode Island Greening apples at 74° F., and the ability of activated sulfuric acid to remove a ripening agent (ehtylene) from these vapors. *A*, Firmness; *B*, carbon dioxide given off per kilogram per hour; *C*, ceric sulfate reduced per kilogram in 20 hours; *D*, aldehyde produced per kilogram in 20 hours. Downward-pointing arrows indicate start of 2-day treatment with apple vapors; upward-pointing arrows, the end of this treatment.

ethylene in the vapors from the ripe Rhode Island Greening apples was not removed below the level of respiratory stimulation when an air flow of 28 liters per hour was employed. The inability of the activated sulfuric acid to absorb the ethylene from the vapors arising

from the postclimacteric fruits in this experiment may have been due to the rapid rate of air flow used.

DISCUSSION

The results of this study indicate that of all the materials tested, brominated, activated charcoal is the one which most nearly approaches the ideal atmospheric washing agent from the standpoint of its ability to remove all the known organic apple emanations. Activated charcoal and the oils tested appear to be ineffective in removing ethylene from the atmosphere surrounding stored apples. Moreover, the oils were very inferior to the activated charcoal in removing the apple emanations that react with concentrated sulfuric acid.

From the evidence presented, it seems reasonable to expect that brominated, activated charcoal would be the most suitable of the materials tested for delaying ripening in storage, but, since the gas or combination of gases liberated by apples which influence the development of scald is not definitely known, it is virtually impossible to predict which, if any, of the materials tested would be suitable for scald control. On the basis of their ability to remove all apple emanations except ethylene, one might expect the oils to be inferior to activated charcoal in controlling scald. Brominated, activated charcoal might prove to be superior to activated charcoal if ethylene is related to the incidence of scald.

No commercial trials have been conducted to ascertain the value of oils, of activated charcoal, or of brominated, activated charcoal as a means of delaying ripening or controlling scald in commercial storage. The value of these materials may depend on the rate and completeness of air circulation within a storage room as well as upon the chemical and physical properties of the substance employed as the atmospheric washing agent. If one is correct in assuming that apple emanations are related to the appearance and severity of scald as well as to the ripening of immature apples, one could hardly expect any materia used to "air-condition" a storage room to be satisfactory unless the organic gases liberated from the fruits are rapidly removed from the atmosphere surrounding the fruits.

SUMMARY

The primary object of this study was to find a suitable material that would react with all the known organic vapors arising from apples.

Activated charcoal was distinctly superior to all the oils tested in its ability to remove vapors of ethyl acetate and the organic apple emanations which react with concentrated sulfuric acid.

The oils, activated charcoal, alkaline potassium permanganate, and activated sulfuric acid were unable to remove the ethylene from postclimacteric apple vapors. At least sufficient quantities of ethylene remained in the air stream after passing through these materials to hasten the appearance of the respiratory and volatile climacteric of immature apples.

Activated charcoal impregnated with bromine was sufficiently reactive at room temperatures to remove ethylene from the vapors arising from ripe apples. The rate of respiration and volatile produc-

tion of preclimacteric apples was not stimulated by the ethylene in ripe apple vapors after the vapors had passed through the brominated, activated charcoal.

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A BIOCHEMICAL STUDY OF SOIL ORGANIC MATTER AS RELATED TO BROWN ROOT ROT OF TOBACCO [1]

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INTRODUCTION

Within the past few years it has become generally recognized that certain crops grown in rotation may have an undesirable effect upon the succeeding crop. Such an effect has frequently been observed on tobacco (*Nicotiana tabacum* L.), and in its most severe form when tobacco has been preceded by a sod crop, particularly timothy. The roots of tobacco growing under these conditions often become discolored or decayed. This condition has led to the use of the term "brown root rot." Previous investigations (*5*)[2] have demonstrated that an organism, *Rhizoctonia bataticola* (Taub.) Butler, is associated with and is a primary cause of brown root rot of tobacco. The present investigation was undertaken to determine whether or not any correlation exists between the occurrence and severity of brown root rot and the biochemical nature of the crop residues which appear to aggravate the disease.

EXPERIMENTAL PROCEDURE

The most obvious means by which a crop could influence a succeeding one would be by the residues of the first crop left in the soil. Such residues were examined biochemically, chiefly by a system of proximate analyses (*7*). The organic fractions investigated included that soluble in benzene-alcohol, the carbohydrate portion, various organic nitrogeneous complexes, and that portion containing lignin, herein designated as lignin-humus. The chemical distribution of soil nitrogen was investigated in a similar manner. The nitrogen fractions included nitrate nitrogen, other water-soluble nitrogen, ammonia nitrogen, amide nitrogen representing acid amides, nitrogen representing amino acids and various heterocyclic compounds, and the nitrogen of organic complexes resistant to prolonged acid hydrolysis. The results were analyzed and interpreted statistically in conjunction with measurements of the severity of brown root rot as it occurred in the soil of the various plots.

ARRANGEMENT AND TREATMENT OF PLOTS

The experimental tobacco plots from which the soils were taken were located at the Tobacco Experiment Station at Upper Marlboro, Md. Since the arrangement of plots, the rotations, fertilizer practices, and yield trends have been given in detail for the most part

[1] Received for publication September 10, 1943. Scientific article No. A66, contribution 1908, of the Maryland Agricultural Experiment Station (Department of Agronomy).

[2] Italic numbers in parentheses refer to Literature Cited, p. 325.

by Garner, Lunn, and Brown (*2*) only a brief description is given here. The soil was mapped as Collington. Most of it was some phase of fine sandy loam, although a few very small areas of loamy sand were found on some plots. The plots chiefly concerned in this paper were the tobacco plots of field IV and field V. The plot arrangement was identical in each field, but field IV and field V bore tobacco in alternate years. Thus field IV was sampled in 1932 and field V in 1933.

These 2 fields were divided into 2 sections of 21 plots each. Each group of 21 plots was divided into 3 groups of 7 plots each. A 2-year rotation of tobacco and grain with different cover crops was used on all plots, except the center plot of each subsection. This plot had a rotation of corn and small grain with no cover crop or fertilizer. The grain crops of the 3 subsections were respectively wheat, oats, and rye. As cover crops, vetch, crimson clover, fallow (kept free of grass and weeds), fallow (corn and small grain plot), cowpeas, soybeans, and grass were used in that order. The grass cover crop was a mixture of timothy, tall meadow oat, orchard, and Italian rye grasses. All except the grasses, which were seeded with the grain crop, were planted after the grain crop and all were plowed under the following spring before tobacco was planted. All crops except corn were fertilized with 60 pounds per acre of P_2O_5 from precipitated dicalcic phosphate and 30 pounds per acre of K_2O from sulphate of potash. Two-thirds of this was applied to tobacco and one-third to the grain crop. None of the plots received nitrogenous fertilizers.

METHODS OF SAMPLING AND ANALYSIS

All plots in field IV were sampled on June 21, 1932, shortly after tobacco had been planted, and were resampled on July 19 and August 23. The corresponding plots in field V were sampled in 1933 on June 18, July 19, and September 17. Because of weather conditions sampling was omitted in August 1933 and a sampling in September was substituted.

Each sample was composited from 20 individual cores of soil. Each core was taken about 10 inches from the plant and about 7 inches from the center of the row. All composite samples were dried at 60° to 65° C., passed through an 8-mesh sieve to remove gravel and the coarser plant material, and then thoroughly mixed.

Nitrate, water-soluble, and ammonia nitrogen were determined on the samples thus prepared. For all other determinations, samples were ground to 40–60 mesh in a Braun pulverizer in order to obtain a more uniform distribution of the organic material in the soil. Nitrate nitrogen was determined by Harper's modification of the phenoldisulfonic acid method (*3*), using a 1 to 5 dilution of the soil. Ammonia nitrogen was estimated in 50-gm. samples by replacement with sodium carbonate according to the aeration method of Matthews (*4*). For water-soluble nitrogen, 100 gm. of soil were shaken at intervals for 30 minutes with 250 ml. of distilled water, filtered through Whatman No. 12 paper, and two 100-ml. aliquots of the filtrate taken for analysis. The Kjeldahl method modified to include nitrate nitrogen was used.

Total nitrogen and total carbon were determined on the pulverized soil. The Kjeldahl method modified to include nitrates was used for total nitrogen. Total carbon was determined by wet combustion, the carbon dioxide being estimated by absorption in standard alkali and

titration of the excess alkali with standard acid. The carbon content of the soil, multiplied by the factor 1.724, was taken as a measure of the total organic matter (*7*).

Further fractionation of nitrogen and organic matter was carried out according to a modification of the proximate system of Waksman and Stevens (*7*), as follows: 100-gm. samples of the pulverized soil were extracted overnight with 1:1 benzene-95 percent alcohol in Soxhlets. The extracts were evaporated and organic carbon of the residues determined by wet combustion. Using the factor 1.724, this fraction represented the fatty, waxy, and resinous materials in the soil organic matter. The extracted soils were carefully dried, transferred to 800-ml. Kjeldahl flasks, and saturated with cold 80 percent sulfuric acid for 2 hours. Fifteen volumes (525 ml.) of distilled water were then added to each flask and the contents of the flasks thoroughly shaken. The flasks were then connected to reflux condensers. Hydrolysis was carried out for 5 hours, after which the digests were filtered through weighed papers, and the residues washed thoroughly with distilled water.

The combined filtrates and washings were made up to 1 liter volume. Reducing sugars were determined on 200-ml. aliquots. After neutralization of aliquots with sodium hydroxide, the resultant sesquioxide precipitates were removed by filtration and washing. The filtrates plus washings were evaporated to small volume and the excess sodium sulphate removed from solution by adding three volumes of 95 percent ethyl alcohol, filtering, and washing with 70 percent alcohol. Alcohol was removed by successive evaporation and addition of distilled water and the solutions finally made up to volume. During the entire process, a chlorophenol red indicator solution was used to guard against an alkaline reaction. Reducing sugars in the prepared solutions were determined by the official permanganate method (*1, p. 192*) and the results were calculated and expressed as cellulose.

Ammonia nitrogen was determined in 100-ml. aliquots of the hydrolysate by distillation with magnesium oxide. The ammonia was taken as a measure of the acid amide content of the soil organic matter (*7*). Total nitrogen determinations on 100-ml. aliquots included not only this ammonia (amide) nitrogen, but also all of the nitrogen rendered soluble by sulphuric acid hydrolysis. The nonamide (total hydrolyzed minus amide) nitrogen represented various amino acids and heterocyclic compounds (*7*) which make up certain organic nitrogenous complexes.

Following the assumption of Waksman and Stevens (*7*) the "protein" or organic nitrogenous complexes of the soil organic matter were calculated by multiplying the percentages of nitrogen thus hydrolyzed by the factor 6.25 and dividing the result by the percent organic matter of the original soil, which was obtained by multiplying the percent carbon by the equally arbitrary factor 1.724. No discussion of the assumed factors will be made here, the subject having been considered in detail elsewhere (*7*).

Residues from hydrolysis of the 100-gm. samples of soil were carefully dried and weighed and total carbon determined on aliquot portions. Again using the factor 1.724 the residual organic matter was calculated. Aliquots also were analyzed for total nitrogen and the results (expressed on the basis of the original soil) multiplied by 6.25. This was

considered to be a measure of the residual organic nitrogenous complex. The difference between this fraction and the residual organic matter was designated as lignin-humus, since it consisted of lignin and its transformation products and various other microbial complexes (7).

STATISTICAL CONSIDERATIONS

For statistical analysis of the effects of cropping systems on the occurrence and severity of brown root rot, the corn plots and the plots without cover crops were omitted. The severity of brown root rot was estimated by direct examination of the tobacco plants in the field, and a root rot index of arbitrarily assigned numbers was established to indicate the severity of the disease, as, trace, 1; mild, 2; moderate, 3; heavy, 4; severe, 5; and most severe, 6.

ANALYTICAL DATA

Table 1 gives the data for total organic matter in the soil, and its proximate composition as fatty-waxy, carbohydrate, nitrogenous, and lignohumic compounds. Table 2 gives the data for total soil nitrogen and its distribution by various fractions. Root rot indices, as measures of the severity of the disease, are included in each of tables 1, 2, and 3. The coefficients of correlation between these index numbers and the various fractions of organic matter and nitrogen were calculated. The values used in determining these coefficients were those used in obtaining the averages given in tables 1 and 2.

The coefficient of correlation between brown root rot and soil organic matter is -0.368 ± 0.091, which is significant. However, analysis of variance indicates that the percentage of organic matter in the soil does not vary with the cover crop to any significant extent. Thus it would appear that it is only coincidence that severe brown root rot occurred in plots containing a small amount of organic matter. This observation is confirmed by the small amount of organic matter in the fallow-rotation plots which were also very low in organic matter and upon which there was no brown root rot.

The correlation coefficients between the various fractions of organic matter and the severity of brown root rot are significant in some cases. They are invariably negative in sign, and appear upon observation to be influenced by the level of total organic matter. That is, in general, any given fraction was low or high on any given plot as the total organic matter of the plot was low or high. By using data for the organic fractions expressed as percentages of the total, this influence of the amount of organic matter was removed and different correlation coefficients were found. These are the coefficients given in table 1. Only two are barely significant: that for the portion of the organic nitrogenous complexes calculated from the nonamide hydrolyzed nitrogen, and that for the lignin-humus fraction. These are $-.213 \pm 0.101$ and $+0.207 \pm 0.101$, respectively.

TABLE 1.—*Average organic matter content of soils of tobacco rotation plots and its proximate percentage composition as carbonaceous, nitrogenous, and carbohydrate compounds*

2-year rotation	Cover crop	Root rot index	Total organic matter in soil	Biochemical composition of soil organic matter								
				Benzene-alcohol soluble fraction	Carbohydrate fraction (as cellulose)	Organic nitrogenous complexes					Lignin-humus	Total organic matter accounted for
						Hydrolyzed nitrogenous complexes			Nitrogen complex insoluble after acid hydrolysis	Total organic nitrogen complexes		
						Amide nitrogen complex	Non-amide nitrogen complex	Total hydrolyzed nitrogen complexes				
			Percent	*Percent of total*	*Percent of total*	*Percent of total*	*Percent of total*	*Percent of total*	*Percent of total*	*Percent of total*	*Percent of total*	*Percent*
Tobacco and wheat	Vetch	2.5	0.7501	5.29	6.53	8.22	15.16	23.38	15.96	39.34	41.07	92.23
Do	Clover	2.0	0.8185	5.30	7.70	10.17	15.81	25.98	17.22	43.20	41.36	97.56
Do	None	1.0	0.6974	4.96	6.94	7.38	14.16	21.54	15.63	37.17	41.93	91.00
Corn and wheat	do	1.0	0.7024	5.68	7.62	6.62	13.74	20.36	16.09	36.45	41.55	91.30
Tobacco and wheat	Cowpeas	1.5	0.8221	5.54	7.98	8.46	15.49	23.95	15.72	39.67	41.92	95.11
Do	Soybeans	1.5	0.8324	5.82	7.32	7.40	14.97	22.37	14.29	36.66	40.92	90.72
Do	Grass	4.0	0.7469	4.89	8.05	7.82	14.38	22.20	14.92	37.12	42.01	92.07
Tobacco and oats	Vetch	2.0	0.7683	4.68	7.49	9.26	18.01	27.27	17.64	44.91	40.20	97.28
Do	Clover	1.5	0.7971	5.28	8.59	8.79	16.78	25.57	17.37	42.94	40.33	97.14
Do	None	1.0	0.6144	5.43	6.51	8.69	15.73	24.42	17.34	41.76	41.38	95.08
Corn and oats	do	1.0	0.5844	5.51	7.28	8.60	14.85	23.45	15.71	39.16	45.19	97.14
Tobacco and oats	Cowpeas	1.0	0.7521	4.72	7.87	7.51	17.09	24.60	17.16	41.76	41.36	95.71
Do	Soybeans	1.0	0.7121	5.50	7.16	8.64	16.31	24.95	14.85	39.80	40.75	93.21
Do	Grass	3.5	0.6523	4.73	9.06	6.97	14.80	21.77	15.53	37.30	42.52	93.61
Tobacco and rye	Vetch	2.5	0.6803	5.46	6.94	8.78	15.84	24.62	15.07	39.69	42.10	94.19
Do	Clover	1.5	0.7276	5.22	6.51	8.24	14.59	22.83	14.56	37.39	42.91	92.03
Do	None	1.0	0.5476	5.72	5.61	6.44	14.20	20.64	15.34	35.98	43.76	91.07
Corn and rye	do	1.0	0.5076	5.17	6.97	7.50	18.69	21.19	16.17	37.36	43.58	93.08
Tobacco and rye	Cowpeas	1.5	0.6531	5.20	7.35	7.12	14.98	22.10	16.31	38.41	39.43	90.39
Do	Soybeans	1.0	0.6595	4.94	7.29	7.86	15.03	22.89	16.32	39.21	41.23	92.67
Do	Grass	4.5	0.6200	5.73	8.02	7.52	14.98	22.50	15.09	37.59	41.36	92.70
Correlation (r) with root rot index [2]			−0.368	+0.040	−0.003	+0.026	−0.213	−0.151	+0.020	−0.107	+0.207	
Standard error			±0.091	±0.105	±0.105	±0.105	±0.101	±0.103	±0.105	±0.104	±0.101	

[1] Brown root rot absent; these plots not included in statistical analysis.
[2] These correlations calculated from original values of which the figures in this table are averages.

TABLE 2.—*Biochemical distribution of nitrogen in the soils of tobacco rotation plots*

2-year rotation	Cover crop	Root rot index	Total nitrogen content of soil	Biochemical distribution of soil nitrogen								
				Nitrate nitrogen fraction	Water-soluble fraction other than nitrate	Ammonia nitrogen fraction	Nitrogen in organic complexes					
							Sulfuric acid hydrolyzable nitrogen			Nitrogen fraction insoluble after acid hydrolysis	Total nitrogen in organic complexes	Total nitrogen accounted for
							Amide nitrogen fraction	Non-Amide nitrogen	Total hydrolyzable fraction			
			Percent	*Percent of total*	*Percent of total*	*Percent of total*	*Percent of total*	*Percent of total*	*Percent of total*	*Percent of total*	*Percent of total*	*Percent*
Tobacco and wheat	Vetch	2.5	0.0535	4.18	2.50	3.08	18.37	33.79	52.16	35.34	87.50	97.26
Do	Clover	2.0	0.0652	5.28	1.66	2.83	20.17	31.53	51.70	34.19	85.89	95.66
Do	None	[1] 0	0.0465	1.00	1.69	2.84	17.79	33.60	51.39	37.34	88.73	94.26
Corn and wheat	do		0.0446	0.63	1.66	3.14	16.49	34.46	50.95	40.13	91.08	96.51
Tobacco and wheat	Cowpeas	1.5	0.0561	1.72	1.82	3.04	18.53	34.98	53.51	36.98	90.49	97.07
Do	Soybeans	1.5	0.0525	2.03	2.42	3.28	18.62	36.87	55.49	36.29	91.78	99.51
Do	Grass	4.0	0.0477	0.59	1.90	3.20	19.30	36.31	55.61	37.01	92.62	98.31
Tobacco and oats	Vetch	2.0	0.0609	4.33	2.79	3.34	18.43	33.73	52.16	35.24	87.40	97.86
Do	Clover	1.5	0.0613	6.05	1.12	4.28	17.93	36.47	54.40	35.86	90.26	101.71
Do	None	[1] 0	0.0437	0.97	1.54	2.86	19.00	35.08	54.08	38.59	92.67	98.04
Corn and oats	do		0.0395	0.66	1.68	2.78	20.31	34.67	54.98	36.78	91.76	96.88
Tobacco and oats	Cowpeas	1.0	0.0518	1.42	1.38	2.27	17.43	39.39	56.82	39.88	96.70	101.77
Do	Soybeans	1.0	0.0466	1.85	1.69	2.87	21.31	38.99	60.30	34.76	95.06	101.47
Do	Grass	3.5	0.0403	0.66	2.27	3.49	18.26	38.14	56.40	40.48	96.88	103.30
Tobacco and rye	Vetch	2.5	0.0480	5.27	1.38	3.21	19.92	35.69	55.61	33.63	89.24	99.10
Do	Clover	1.5	0.0481	4.85	0.42	2.94	20.15	35.88	56.03	35.77	91.80	100.01
Do	None	[1] 0	0.0334	0.94	1.02	4.87	16.44	36.72	53.16	40.57	93.73	100.56
Corn and rye	do		0.0319	0.63	1.08	4.66	18.84	35.33	54.17	41.12	95.29	101.66
Tobacco and rye	Cowpeas	1.5	0.0432	1.36	0.78	3.44	17.24	36.78	54.02	39.80	93.82	99.40
Do	Soybeans	1.0	0.0434	1.59	0.96	3.25	18.69	35.43	54.12	39.09	93.21	99.01
Do	Grass	4.5	0.0410	0.74	2.04	3.14	17.95	37.25	55.20	37.36	92.56	98.48
Correlation (r) with root rot index [2]			−0.386	−0.273	−0.039	−0.009	+0.152	−0.187	−0.092	+0.166	+0.039	
Standard error			±0.090	±0.097	±0.105	±0.105	±0.103	±0.102	±0.105	±0.103	±0.105	

[1] Brown root rot absent; these plots not included in statistical analysis.
[2] These correlations calculated from original values of which the figures in this table are averages.

TABLE 3.—*Rotations, occurrence and severity of brown root rot, and carbohydrate: nitrate nitrogen ratios of tobacco rotation plots*

2-year rotation	Cover crop	Root rot index	Carbohydrate: nitrate nitrogen ratio
Tobacco and wheat	Vetch	2.5	26.3
Do	Clover	2.0	28.7
Do	None	[1] 0	
Corn and Wheat	do		
Tobacco and wheat	Cowpeas	1.5	81.8
Do	Soybeans	1.5	53.5
Do	Grass	4.0	194.1
Tobacco and oats	Vetch	2.0	32.8
Do	Clover	1.5	33.9
Do	None	[1] 0	
Corn and oats	do		
Tobacco and oats	Cowpeas	1.0	94.9
Do	Soybeans	1.0	66.0
Do	Grass	3.5	267.5
Tobacco and rye	Vetch	2.5	37.2
Do	Clover	1.5	41.1
Do	None	[1] 0	
Corn and rye	do		
Tobacco and rye	Cowpeas	1.5	94.7
Do	Soybeans	1.0	99.0
Do	Grass	4.5	221.2
Correlation (r) with root rot index [2]			$+0.479$
Standard error			± 0.081

[1] Brown root rot absent; these plots not included in statistical analysis.
[2] This correlation calculated from original values of which the figures in this table are averages.

The coefficient of correlation between the severity of brown root rot and the total nitrogen content of the soil was found to be -0.386 with a standard error of ± 0.090, which is significant (table 2). Analysis of variance indicates that the total nitrogen content varied significantly with the cover crop. Correlations between the various fractions of nitrogen, expressed as parts per million of the whole soil and the severity of brown root rot are all negative. Although some appear to be significant, they are obviously associated with variations in the total nitrogen content of the soil. The influence of this total nitrogen is overcome by a redetermination of the coefficients using the nitrogen fractions expressed as percentages of the total. These coefficients are given in table 2.

The only significant coefficient of correlation in table 2 is that between the severity of brown root rot and the nitrate nitrogen fraction, -0.273 ± 0.097. Since this coefficient is negative it is evident that low amounts of nitrate nitrogen are associated with severe brown root rot. Analysis of variance shows that variation in nitrate nitrogen with the cover crop preceding tobacco was overwhelmingly significant. However, the fallow plots on which brown root rot did not occur were quite low in nitrate nitrogen.

It will be noted in table 1 that the carbohydrate fraction of the soil was of no significance in its relation to brown root rot. If, however, values for the first sampling of each season are employed, the correlation with brown root rot is $+0.311 \pm 0.165$. Although this correlation is not quite significant, it is interesting to note that at least in a general way a high percentage of carbohydrate in the organic matter during the early season was associated with greater severity of brown root rot.

The simple coefficient of correlation between the carbohydrate fraction and nitrate nitrogen was found to be $+0.360 \pm 0.092$. This correlation is based upon carbohydrate and nitrate nitrogen expressed as parts of the whole soil, and not as parts of the total organic matter and total nitrogen, respectively. The whole soil is the only common basis for those two factors.

Using this correlation coefficient, the partial correlation between the severity of brown root rot and the carbohydrate fraction, with the influence of the nitrate factor constant, was found to be -0.092, which of course is nonsignificant. However, when brown root rot is correlated with nitrate nitrogen, with the influence of the carbohydrate factor constant, the partial coefficient of correlation becomes -0.245. This is significant although a little less so than the simple correlation between brown root rot and nitrate nitrogen. This correlation might indicate that although the carbohydrate fraction might be significant in influencing the nitrate content of the soil, it had very little direct bearing upon brown root rot. The multiple correlation is more significant, being $+0.305 \pm 0.096$, greater than either of the simple correlations involved. This indicates that variations in the severity of brown root rot are associated with variations in these two soil constituents.

In a further study of these two constituents, the carbohydrate: nitrate nitrogen ratio for each soil was calulcated (table 3). In making these calculations it was impossible to construct ratios where analyses showed nitrate nitrogen to be absent from the soil. To expedite the work, the few soils (four in number) that contained no nitrate nitrogen were arbitrarily assigned 1 part per million of nitrate in order that ratios other than infinity could be constructed.

The correlation between the severity of brown root rot and the carbohydrate: nitrate nitrogen ratios thus constructed was found to be $+0.479 \pm 0.081$. This correlation is highly significant, and indicates strongly that the more severe brown root rot was associated with wide carbohydrate : nitrate nitrogen ratios.

To determine whether the carbohydrate or the nitrate in themselves influenced the correlation between brown root rot and the carbohydrate: nitrate nitrogen ratio, partial correlation coefficients were calculated. When the severity of brown root rot was correlated with the ratio, with nitrate nitrogen constant, the severity of brown root rot still varies directly with the carbohydrate: nitrate nitrogen ratio, although not so significantly as in simple correlation. When brown root rot was correlated with the ratio, with the carbohydrate fraction constant, the partial correlation coefficient was $+0.492$. With the carbohydrate content of the soil constant, the severity of brown root rot apparently would be even more highly correlated with the carbohydrate: nitrate nitrogen ratio. Thus of the two factors involved, nitrate nitrogen played by far the more important role in the severity of brown root rot, and the carbohydrate content was of secondary importance.

DISCUSSION

As was pointed out above, the negative correlation between organic matter and the severity of brown root rot seems to be only coincidental. This seems to be confirmed by the fact that although brown root rot

varied significantly with the preceding cover crop, the total organic content of the soil did not. The fallow plots, in which the organic content was very low, did not produce brown root rot. In previous work (*5*) brown root rot was most severe when cellulose was added, increasing the organic content of the soil. These points are definite evidence that brown root rot does not increase with decreasing organic matter in the soil, and that greater severity of brown root rot in the field is only coincidental with low organic matter.

It is noted that only two fractions of the organic matter, as determined by the system of proximate analysis employed, are correlated at all significantly with the severity of brown root rot. Those fractions are the lignin-humus and that part of the organic nitrogenous complexes calculated from nonamide hydrolized nitrogen. If highly significant, which they were not, the correlations would indicate that large percentages of nonamide complexes accompany slight severity of brown root rot, and that large percentages of lignin-humus in the organic matter accompany great severity of brown root rot. The correlations are too small to justify anything more than the barest generalizations, even though statistically they are significant.

Although a significant coefficient of correlation (-0.386 ± 0.090) is found between the total nitrogen content of the soil and the severity of the disease, and although analysis of variance shows that total nitrogen varies significantly with the previous cover crop, there are facts which minimize the importance of the total nitrogen of the soil. (1) The plots upon which no cover crops were grown (omitted from statistical analysis) and which produced no brown root rot, contained even less total nitrogen than the plots on which brown root rot was most severe. (2) The correlation of brown root rot with total organic matter probably was coincidental. Since total nitrogen is highly dependent upon and correlated with total organic matter, it follows that the variations in the percentage of total nitrogen in the soil are also probably coincidental with variations in the severity of the disease. (3) These factors are so positively correlated with each other that partial correlations with one, holding the influence of the other constant, are nonsignificant. These facts seem to indicate that neither the total organic matter content nor the total nitrogen content of the soil is highly significant in its effect upon the severity of brown root rot of tobacco.

Of the different nitrogen fractions investigated, the only association that is significant is that with nitrate nitrogen. Analysis of variance shows that the association between the nitrate nitrogen fraction and the kind of preceding cover crop is highly significant. The fact that nitrate nitrogen was low on fallow plots, which produced no brown root rot, seems to indicate, however, that large amounts of nitrate nitrogen are not necessary to prevent the disease, and that some other factor is probably involved. This other factor appears to be the carbohydrate fraction of the soil organic matter. As pointed out, the association with carbohydrate content of the organic matter is almost significant when only the first early season samplings are considered. Also, the increase in value of the multiple correlation coefficient ($+0.305 \pm 0.096$) between brown root rot and both nitrate and carbohydrate over either of the simple correlations, indicates that the carbohydrate association must have some significance. The effect

of the carbohydrate on the correlation between brown root rot and the nitrate content of the soil is shown in the partial correlations. There is a significant correlation (−0.245) between brown root rot and nitrate nitrogen when the influence of the carbohydrate fraction is held constant. Thus the carbohydrate factor does affect nitrate nitrogen, but probably does not affect brown root rot directly.

The importance of nitrate nitrogen as it might influence brown root rot is emphasized by the correlations between brown root rot and the carbohydrate: nitrate nitrogen ratio. Although the simple correlation coefficient is very significant ($+0.479 \pm 0.081$), partial correlations between brown root rot and the ratio, when the influence of the constituent parts, either carbohydrate or nitrate, is held constant, shows that the nitrate nitrogen rather than the carbohydrate is the most important factor influencing the correlation between brown root rot and the carbohydrate : nitrate nitrogen ratio.

No definite minimum value for nitrate nitrogen below which brown root rot should occur, can be designated. However, it would seem that the critical nitrate value, if any, would be dependent in some manner upon the carbohydrate: nitrate nitrogen ratio of the soil. To test this supposition, the partial correlation between the severity of brown root rot and nitrate nitrogen was determined, with the influence of the carbohydrate: nitrate nitrogen ratio held constant. The value was found to be but −0.044, as compared to −0.292 for the simple correlation between brown root rot and nitrate nitrogen, as parts of the whole soil. Evidently the influence of variations in nitrate nitrogen upon the severity of brown root rot is dependent upon the influence of variations in the carbohydrate: nitrate nitrogen ratio of the soil.

The results of this work seem to confirm those obtained by Thomas (*6*) in his earlier work on nitrate nitrogen and nitrification in relation to growth of tobacco and to brown root rot. However, the work of Thomas apparently indicates that carbohydrate material has a more important direct bearing upon the occurrence and severity of the disease than does the present work. The statistical analysis here presented shows that it is highly important only in its ratio to nitrate nitrogen. It shows further that the ratio is of significance chiefly in its influence upon the association between brown root rot and the nitrate nitrogen content of the soil. Nitrate nitrogen is the only soil constituent in itself found to be highly significant in its direct influence upon the severity of brown root rot of tobacco.

SUMMARY AND CONCLUSIONS

A study was made of biochemical soil factors which might influence the disease known as brown root rot of tabacco. A number of soils from tobacco rotation plots at upper Marlboro, Md., were investigated. The amount of organic matter in each was determined as well as the following constituents of the organic matter: The benzene-alcohol soluble fraction, the carbohydrate material, organic nitrogenous complexes as calculated from amide, nonamide acid-hydrolyzed, hydrolysis-resistant nitrogen, and lignin-humus. The total soil nitrogen and its biochemical distribution was determined, the fractions investigated being nitrate nitrogen, water-soluble nitrogen other than nitrate, ammonia nitrogen, amide nitrogen, nonamide acid-hydrolyzed

nitrogen, and resistant or nonhydrolyzed nitrogen. The severity of brown root rot was estimated by direct examination of the tobacco roots, and a root rot index established. The laboratory results were analyzed statistically in conjunction with the root rot indices, in order to determine what constituents of the soil organic matter or what fractions of soil nitrogen might be associated with the severity of brown root rot.

Nitrate nitrogen was the only single soil constituent found to be very significant in its influence upon the severity of brown root rot. The coefficient of correlation between nitrate nitrogen, as parts per million of the soil, and the severity of the disease was found to be -0.292 ± 0.096. When nitrate nitrogen is expressed as percent of the total nitrogen, this coefficient is -0.273 ± 0.097. The carbohydrate fraction alone was of no significance, but the correlation between brown root rot and the carbohydrate: nitrate nitrogen ratio is the most significant encountered, being $+0.479 \pm 0.081$. Partial correlations indicate that the influence of variations in nitrate nitrogen upon the severity of brown root rot was very largely dependent upon the influence of variations in the carbohydrate: nitrate nitrogen ratio.

It is concluded that the severity of brown root rot is associated negatively with the nitrate nitrogen content and with the carbohydrate-nitrate nitrogen ratio of the soil. The preceding crop affects the succeeding tobacco crop indirectly by its influence upon the carbohydrate: nitrate nitrogen ratio, and more directly by its effect upon the nitrate nitrogen content of the soil.

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